Study of Zooplankton Feeding Selectivity by HPLC Analysis of Phytoplankton Pigment

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Abstract

Seasonal change in feeding selectivity of zooplankton in Tolo Harbour was studied using HPLC analysis of phytoplankton pigments. The research consisted of four parts. In the first part, seasonal change in feeding selectivity of *P. avirostris* and *Paracalanus* spp. was studied using HPLC pigment analysis method, cell counting method and gut fluorescence method. Water and zooplankton samples were collected at nearly monthly intervals at two stations in the inner part of Tolo Harbour. Pigment ratios in the water were compared to those in the guts of zooplankton. Any differences indicated the occurrence of zooplankton feeding selectivity. Fluorescence method measuring chlorophyll-*a* concentrations in water and zooplankton samples confirmed the accuracy of the HPLC analysis. The phytoplankton pigments chlorophyll *c3*, peridinin, fucoxanthin, alloxanthin and lutein were used as markers for prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green algae, respectively. *P. avirostris* selected for diatoms, cryptomonads and green algae, but selected against dinoflagellates and prymnesiophytes. Selectivity of cryptomonads, green alga and dinoflagellates exhibited by *P. avirostris* changed seasonally with changes in the composition of the phytoplankton. *P. avirostris* exhibited strong selection for dinoflagellates when the dinoflagellate populations were dominated by *Prorocentrum* spp. Selectivity by *P. avirostris* on dinoflagellates was weak when the most common dinoflagellates were *Scrippsiella* spp. and *Noctiluca scintillans*. *Paracalanus* spp. showed strong selection for diatoms, but selected against prymnesiophytes, dinoflagellates and green alga. Selection on cryptomonads, green alga and prymnesiophytes by *Paracalanus* spp. changed seasonally with changes in the composition of the phytoplankton.

The second part of my research was an investigation on the feeding selectivity of *Pseudevadne tergestina*. *P. tergestina* showed strong selection for diatoms, but selected against prymnesiophytes, dinoflagellates, cryptomonads and green alga.

The third part of my research consisted of a series of laboratory feeding experiments. Zooplankters collected from Tolo Harbour were fed natural phytoplankton and filtered seawater enriched with laboratory cultured chrysophytes. Pigment degradation rates were calculated using the equation of Pasternak (1994) and were used to determine the degree by which *in situ* zooplankton feeding rates on different algal groups had been underestimated by the HPLC pigment analysis technique. Pigment degradation after zooplankton ingestion ranged from 4% for lutein to 33% for chlorophyll *a*. With the verification of the *in-situ* experiment by the laboratory feeding experiments, *in situ* zooplankton feeding rates determined by HPLC technique are more reliable.

Finally, clearance rates for *P. avirostris* and *Paracalanus* spp. on different phytoplankton classes were calculated based on feeding experiments carried out in part three of the research. Mean clearance rates ranged from 0.02 mL h^{-1} (dinoflagellatesi) to 1.02 mL h^{-1} (total phytoplankton) for *P. avirostris* and from 0.01 mL h^{-1} (dinoflagellates) to 1.55 mL h^{-1} (prymnesiophytes) for *Paracalanus* spp. Food selectivity patterns obtained in field samples were supported by the results of laboratory feeding experiments. Grazing rates of *P. avirostris* on diatoms, cryptomonads and green alga were higher than those on dinoflagellates and prymnesiophytes. *Paracalanus* spp. also showed a high clearance rate on diatoms.

摘要

利用高效能液相色層層析儀 (HPLC) 分析浮游植物之色素，對鳥喙尖嘴溞 (*Penilia avirostris*) 及擬哲水蚤屬 (*Paracalanus* spp.) 在吐露港的攝食選擇性季節變化進行了研究。研究共有四個部分。在第一部分，HPLC 色素分層技術，細胞數算法及腸內螢光色素測定法都被用來爲 *P. avirostirs* 及 *Paracalanus* spp. 的攝食選擇性季節變化進行研究。差不多每一個月，在吐露港的內港的兩個站都會收取水和浮游動物的樣本。在水中的色素比例會和浮游動物腸內的色素比例作比較。 任何的不同都顯示了浮動物游攝食選擇性的出現。用螢光色素測定法量度葉綠素甲(chl-*a*) 在水和動物浮游生物樣本中的濃度確定了 HPLC 色素分層技術的準確性。 浮游植物色素葉綠素丙三 (chl-*c3*), peridinin, fucoxanthin, alloxanthin 及 lutein 分別被用作金藻、甲藻、硅藻、隱藻及綠藻的標記。*P. avirostris* 選擇攝食硅藻、隱藻及綠藻，但卻抗拒攝食甲藻及金藻。*P. avirostris* 對隱藻、綠藻及甲藻所作出的選擇伴隨着浮游植物成份的季節性變化而改變。當甲藻以原甲藻屬(*Prorocentrum* spp.) 爲主，*P. avirostris* 顯示了對甲藻強列的選擇性。*P. avirostris* 對甲藻的選擇性卻會在以斯氏藻屬 (*Scrippsiella* spp.) 及夜光溞 (*Noctiluca scintillans*) 爲多數甲藻的時候變弱。擬哲水蚤屬 (*Paracalanus* spp.) 對硅藻顯示了強列的選擇性，但對金藻、甲藻及綠藻就抗拒選擇。擬哲水蚤屬 (*Paracalanus* spp.) 對隱藻、綠藻及金藻的選擇隨著浮游植物成份的季節性轉變而變化。

第二部份的研究是一個對肥胖三角溞 (*Pseudevadne tergestina*) 攝食選擇性

的研究。*P. tergestina* 顯現了對硅藻的強列選擇，卻抗拒選擇金藻、甲藻、隱藻及綠藻。

第三部份的研究包括一系列的實驗室攝食實驗。於吐露港收集的浮游動物被餵飼天然浮游植物及在實驗室繁殖的金黃藻。色素降解率通過 Pasternak (1994) 的方程式被計算，同時也被用作確定天然情況下水中浮游動物對不同種類的藻的攝食率被 HPLC 色素分層技術低估的程度。浮游動物攝食後的包素降解由對 lutein 的 4% 至葉綠素甲 (chl-*a*) 的 33%。野外實驗得到實驗室攝食實驗的複刻，結果將會更可信。

最後，根據在研究第三部份進行過的攝食實驗，*P. avirostris* 及 *Paracalanus* spp. 對不同浮游植物的攝食速度被計算。平均攝食速度範圍由 *P. avirostris* 的 0.02 mL h^{-1} (甲藻)到 1.02 mL h^{-1} (整體浮游植物)和 *Paracalanus* spp. 的 0.01 mL h^{-1} (甲藻)到 1.55 mL h^{-1} (金藻)。從野外樣本得到的食物選擇性模式被實驗室攝食實驗結果支持。*P. avirostris* 對硅藻 隱藻和綠藻的攝食速度比對甲藻和金藻的高。*Paracalanus* spp. 同樣表現了一個對硅藻高的攝食速度。

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CHAPTER 1 INTRODUCTION

Feeding selectivity of herbivorous zooplankton is important in affecting the structure and composition of the phytoplankton community (Bergquist *et al.*, 1985). For example, if the zooplankton only eats phytoplankton that is rare, the amount of energy transferred to the upper tropical levels will be small. The energy in the common phytoplankton species will go to the decomposers. The efficiency of trophic transfer will be low. In areas where water is shallow and circulation is poor, decomposition of uneaten phytoplankton may result in anoxia of the bottom layers. In some cases, selective grazing of zooplankton may cause blooms of ungrazed phytoplankton species. For example, Riegman *et al.* (1993) showed that large diatoms bloomed in the Marsdiep area in the Netherlands because the large diatoms were too big to be fed on by microzooplankton which governed the abundance of small-sized phytoplankton. Also, Gasparini *et al.* (2000) showed that the low grazing pressure of the dominant copepod species on *Phaeocystis globosa*, together with the preferential grazing on diatoms which reduces the competition for nutrients, and the predation of the dominant copepod species on microzooplankton organisms which reduces the microzooplankton grazing pressure on *P. globosa* cells, were likely to favour the *P. globosa* bloom in the Southern Bight of the North Sea. These findings suggest that grazing by zooplankton may be a control of phytoplankton bloom. Since zooplankton was important in marine food webs (Hardy, 1924; Ryther, 1969; Lasker, 1988) and in the geochemical transformations, many studies were carried out on the foraging behavior (Alcaraz *et al.*, 1980; Kiørboe *et al.*, 1982; Koehl & Strickler, 1982; Cowles & Strickler, 1983; Price *et al.*, 1983), feeding rates (Frost, 1972; Paffenhöfer & Harris, 1976) and foods (Easterly, 1916; Schnack & Elbrächter, 1983; Turner, 1984a, b, c; Stoecker & Sanders, 1985; Huntley & Boyd, 1984; Kleppel, 1992, 1993; Poulet *et al.*, 1994; Jónasdóttir *et al.*, 1995; Kleppel *et al.*, 1996) of zooplankton.

The concept of selectivity assumes that the feeding behavior of animals could vary in relation to the type of food available (Knisely and Geller 1986; Borsheim and Andersen 1987; Head and Harris 1994)

Since feeding selectivity of zooplankton is so important, feeding selectivity of zooplankton in Tolo Harbour was studied in my MPhil research. Tolo Harbour is an embayment located in the northeast of Hong Kong. Four species of marine cladocerans (*Penilia avirostris*, *Pseudevadne tergestina*, *Podon schmackeri* and *P. polyphemoides*) out of the total 8 species in the world can be found in Tolo Harbour. Information on the feeding selectivities of cladocerans was limited. Therefore, Tolo Harbour is an ideal location for studying the feeding ecology of marine cladocerans. In addition, Tolo Harbour accounts for almost 50% of the red tides that occurred in Hong Kong (HKEPD, 2000). Studying feeding selectivity of zooplankton in Tolo Harbour can give a better understanding of the ability of zooplankton to control red tides and also the possible reasons leading to the red tide blooms. In my research, the marine cladocerans *P. avirostirs* and *P. tergestina* were chosen for study. *P. avirostris* is frequently abundant in warm and productive nearshore waters of the tropics and subtropics (Della Croce & Venugopal, 1972; Grahame, 1976; Moore & Sander, 1979). In temperate waters, *P. avirostris* exists seasonally in large numbers in polluted estuaries (Yoo & Kim, 1987). In the subtropical coastal waters of Hong Kong, *P. avirostris* occurs sporadically throughout the year and occasionally forms an important component of the crustacean zooplankton assemblage (Chen, 1982; Chan, 1991). Kim et al. (1989a) noted that marine cladocerans may play an important role in the trophodynamic pathways of the plankton community in the Inland Sea of Japan during periods of high abundance. *P. avirostris* could also be an important food for carnivorous zooplankton, pelagic fish and fish larvae (Cheng & Chao, 1982). *P. tergestina* was abundant in winter in Tolo Harbour (Tang *et al.*,

1995). In addition to the two cladoceran species, the calanoid copepods *Paracalanus* spp. were studied. Wong *et al.* (1993) found that *Paracalanus* spp. occurred in high abundance in Tolo Harbor throughout the year. Therefore, as the most abundant herbivores in Tolo Harbour, *Paracalanus* spp. may influence the abundance and composition of the phytoplankton community.

Traditional methods to study feeding selectivity of herbivorous zooplankton include microscopic cell counting to determine the change in the number of phytoplankton cells before and after grazing, gut content examination with both light microscopes and scanning electron microscopes (SEM), use of radioisotope tracers such as ^{14}C and ^{32}P, and use of fluorescent tracers such as chlorophyll-*a* (Omori & Ikeda, 1984; Harris *et al.*, 2000). However, all of the above methods have their own disadvantages. Cell counting after laboratory feeding experiments raises the possibility of disturbance from laboratory handling. The feeding environment created in the laboratory is never identical to the natural environment. Behavior of zooplankton in the laboratory may be different from the natural behavior (Harris *et al.*, 2000). Microscopic examination of gut contents is also difficult as some algae may be fragile and easily digested (Omori & Ikeda, 1984). In addition, it is difficult to examine a large number of samples. The use of radioisotope tracers on shipboard and in the field poses safety problems. This method cannot provide taxonomic information on the food being eaten by zooplankton. Analysis of pigments such as chlorophyll-*a* by fluorescence method do not give taxonomic information on the ingested phytoplankton. Phytoplankton such as some dinoflagellates that do not contain chlorophyll-*a* cannot be investigated using this method.

Recently, high performance liquid chromatography (HPLC) analysis of phytoplankton pigments is used in studying zooplankton feeding selectivity (Head & Harris, 1994; Swadling & Marcus, 1994; Buffan-dubau *et al.*, 1996; Kleppel *et al.*,

1996; Quiblier-Llobéras *et al.*, 1996; Breton *et al.*, 1999; Meyer-Harms *et al.*, 1999; Gasparini *et al.*, 2000; Suzukim *et al.*, 2002). This method uses specific chlorophyll-*a* and carotenoid pigments as chemotaxonomic markers of phytoplankton classes and measures feeding selectivity by determining the concentration of different pigments in zooplankton guts. Therefore, this method can provide class-specific information on the food being eaten by zooplankton. In addition, this method can be applied in *in situ* experiments. The phytoplankton eaten by zooplankton at the time of sampling was frozen for analysis. The results obtained were purely natural without any handling disturbance. The availability of commercial pigment markers also makes this method more accessible. Up to 48 sample runs can be done in one day in my experiments. Therefore, HPLC analysis of phytoplankton pigments was used as the main analyzing method in this research.

Information on the feeding selectivity of *Penilia avirostirs* (Section 2.3.3.1), *P. tergestina* and *Paracalanus* spp. (Section 2.3.3.2) is limited. In my research, the HPLC pigment analysis method was used to study the *in situ* feeding selectivity of these three zooplankters. The HPLC method is limited by the problem of pigment degradation. Phytoplankton pigments in zooplankton guts may be digested. The rates of digestion for different phytoplankton pigments may also vary. Therefore, the amounts and ratios of different phytoplankton pigments in zooplankton guts may be underestimated and inaccurate respectively. Pigment degradation experiments have been reported by many researchers (Lopez & Huntley, 1988; Penry & Frost, 1991; Head, 1992; Mayzaud & Razouls, 1992; Head & Harris, 1992, 1994; Suzanne *et al.*, 1998). Pigment degradation was found to vary with the food concentration, feeding history and species of the zooplankton. Experiments were carried out in my research to study pigment degradation in zooplankton gut samples.

The objectives of my MPhil research are to use the HPLC pigment analysis method to: (1) study seasonal patterns in feeding selectivity of the marine cladoceran *P. avirostirs* and the marine copepod *Paracalanus* spp. in Tolo Habour; (2) investigate feeding selectivity of other marine cladocerans including *P. tergestina*; (3) estimate the degree of pigment degradation after ingestion by zooplankton in order to more accurately estimate the amount of phytoplankton eaten by zooplankton as determined by the HPLC pigment analysis method; and (4) estimate the clearance rates of *P. avirostris* and *Paracalanus* spp. on different classes of phytoplankton in laboratory feeding experiments.

In the first studies, zooplankton and phytoplankton samples in Tolo Harbour were sampled nearly monthly from February 2003 to January 2004 (except July and September 2003). Feeding selectivity of *P. avirostris* and *Paracalanus* spp. were investigated using HPLC analysis of phytoplankton pigments. Ratios of different phytoplankton pigments (chlorophyll *c3*, peridinin, fucoxanthin, alloxanthin and lutein) in water samples and zooplankton guts were compared. Difference in these ratios indicated the occurrence of zooplankton feeding selectivity. This method was also compared with fluorescence method in which concentration of chlorophyll-*a* in water obtained by HPLC analysis and fluorescence method were compared. Besides, phytoplankton samples collected were also examined by cell counting method using light microscope. Abundances of different phytoplankton classes were determined. Dinoflagellates were identified to the genus level to provide information on feeding selectivity at the genus level. Finally, zooplankton communities and structures were examined using dissecting microscope.

In the second study, feeding selectivity of the podonid cladoceran *P. tergestina* was investigated in several occasions when dense populations of this species were encountered in Tolo Harbour. Literature information on the feeding selectivity of

podonids is limited. Results obtain in this part of my research is important to planktologists. In the third study, a series of laboratory feeding experiments were done using zooplankton and phytoplankton collected in Tolo Harbour. Laboratory cultured chrysophytes were also used as feeding medium. Experiments were carried out on 3 and 30 July 2003, 2 October 2003, 4 August 2003 (for *Paracalanus* spp. only), 6 November 2003 and 1 December 2003. Phytoplankton communities before and after feeding experiments were analyzed by HPLC for phytoplankton pigments and by light microscopy for cell densities. Phytoplankton pigment degradations after ingestion by *P. avirostris* and *Paracalanus* spp. were calculated using the equation of Pasternak (1994). After zooplankton ingestion, phytoplankton pigments in zooplankton guts may be digested. The rates of digestion for different phytoplankton pigments may also vary. Pigment degradation may affect the amounts and ratios of different phytoplankton pigments in zooplankton guts. Complete digestion of ingested phytoplankton may lead to wrong conclusion about feeding selectivity by zooplankton. Therefore, my experiments were done to determine the applicability of the HPLC pigment analysis method in zooplankton feeding studies.

In the forth study, clearance rates of *P. avirostirs* and *Paracalanus* spp. on different phytoplankton classes were measured in the laboratory. High clearance rate indicated preference of the phytoplankton class. Comparison with results obtained in *in situ* experiments gave more evidences on the zooplankton feeding selectivity.

CHAPTER 2 LITERATURE REVIEW

2.1 Traditional methods for studying zooplankton feeding selectivity

2.1.1 Cell counting after laboratory feeding experiments

Traditionally, investigation of zooplankton feeding selectivity was based on laboratory feeding experiments. Densities of phytoplankton cells in the feeding medium were determined by microscopic counting before and after the feeding incubation (Bergquist et al., 1985; Knisely & Geller, 1986; Scheda & Cowell, 1988). The difference between the two reflects the food eaten by the zooplankton. However, experiments must be carried out under artificial conditions in the laboratory or on board ship. In addition, phytoplankton counting is time consuming, especially if statistically valid counts of the less abundant plankton classes are required. Also, smaller phytoplankton, especially the picoplankton, can be difficult to identify since they lack taxonomically useful external morphological features (Mackey *et al.*, 1979). The increased resolution of scanning or transmission electron microscopy allows identification of the picoplankton, but sample preparation requirements render electron microscopy extremely time-consuming for phytoplankton identification in large-scale surveys (Mackey *et al.*, 1996). Many species are very fragile and do not survive sample fixation (Gieskes & Kraay 1983). Epi-fluorescence microscopy (Waterbury *et al.*, 1979) and analytical cell-flow cytometry (Chisholm *et al.*, 1988) allow detection of chlorophyll-*a* and biliprotein autofluorescence in single cells and enable the discovery of minute prochlorophytes and cyanobacteria. However, if the disadvantage of laboratory disturbance is not considered, the cell counting method is an accurate method. Many researchers include this method in their studies in addition to other methods to give more evidences on the feeding selectivity of zooplankton (Kleppel, 1996; Meyer-Harms, 1999; Gasparini, 2000). In my study, the cell counting method was also used to

overcomesome of the shortcomings of the HPLC phytoplankton pigment analysis method.

2.1.2 Direct examination of gut contents

Gut contents of zooplankton collected in the field are examined under light microscope or electron scanning microscope (Ferguson *et al.*, 1982; Toth & Zankai, 1985; Kim *et al.*, 1989). Some phytoplankton groups are fragile and easily digested. Therefore, the assumption that phytoplankton groups that are not found in zooplankton guts are not eaten by zooplankton may not be true, especially for food items that are easily digested. In addition, in the course of sampling zooplankton with a net, large zooplankton may swallow small zooplankton and other material concentrated in the cod-end of the net. This artifact, called net feeding, occurs frequently when smaller mesh nets are used to collect large zooplankton species. For example, Angel (1970) reported that the gut of an ostracod, *Conchoecia spinirostris*, was packed with copious quantities of freshly eaten, black pigmented tissue originating from myctophid fishes caught in the same net haul. Furthermore, identifiable material such as diatom frustules may originate from either materials ingested by the predator of interest or from food ingested by its prey (Omori & Ikeda, 1976). Example of research investigating the food of zooplankton using this method includes gut content examination of marine cladocerans by Kim *et al.* (1989). They used scanning electron microscope to examine the guts of five marine cladocerans. Centric diatoms with a few exceptions of pennate diatoms & dinoflagellates were found.

2.1.3 Use of radioactive tracers

Use of radioisotope tracers in studying zooplankton feeding selectivity involves labeling of different classes of phytoplankton with radioisotopes. These labeled phytoplankton are then fed to the zooplankton in the laboratory or on board ship. Clearance rates of zooplankton on different classes of phytoplankton can be calculated by tracing the magnitudes of radioactive isotopes increased in zooplankton gut or the decrease in magnitudes in the feeding medium. This method is sensitive. However, the following problems may exist. (1) Differential uptake of labeled compounds may occur. (2) Incubation and handling of the animals may change the animals' behaviors. (3) Safety problems may result when experiments are conducted in the field or on the ship. Some investigators used this method in addition to the HPLC method. For example, Gasparini *et al.* (2000) labeled four types of phytoplankton and then fed to zooplankton. DPM (Disintegration Per Minutes) in the food medium before and after feeding was quantified. Clearance rates for the different types of phytoplankton were calculated as a high clearance rate indicated a preference of that kind of phytoplankton.

2.1.4 Gut fluorescence method

In the gut fluorescence method, the amount of chlorophyll-*a* and its derived pigments in the gut of zooplankton is measured with a fluorometer (Nemoto, 1968; Nemoto & Saijo, 1968). The unique photosynthetic pigment, chlorophyll-*a*, has been used as a simple biomass indicator of marine phytoplankton since the early 1950s. The amount of pigment can reflect the amount of phytoplankton eaten. However, as nearly all phytoplankton contain chlorophyll-*a*. This method cannot provide taxonomic information and thus no information on food selectivity. In addition, pigment degradation may occur. The fluorometric method tends to underestimate

chlorophyll-*a* when chlorophyll *b* is abundant in the water (Li *et al.*, 2002; Gieskes, 1991). Earlier investigations of this method has shown that its accuracy depended on the sample matrix and the calculation equations used (Lorenzen & Jeffrey, 1980; Wright & Jeffrey, 1997). Upgraded chlorophyll methods of varying accuracy had been used since then in oceanography (see Jeffrey and Mantoura 1997).

2.2 High Performance Liquid Chromatography (HPLC) analysis of phytoplankton pigments

2.2.1 Principle

HPLC analysis of phytoplankton pigments makes use of the differences in polarity and molecular size of different phytoplankton pigments. Because of these differences, different phytoplankton pigments have different retention times. That is, different phytoplankton pigments have a different solubility on the solvents running in the column and then come out from the column at different times. As a result, different phytoplankton pigments can be separated. The integration of the area under a particular peak in absorbance or fluorescence at 436 nm or 640 nm respectively is a measure of the amount of pigment injected into the column. They can then be qualified and quantified with a standard pigment.

In the study of zooplankton feeding selectivity using HPLC analysis of phytoplankton pigments, different phytoplankton pigments to total pigment ratios in both water and zooplankton guts are obtained. By comparing these ratios, the occurrence of zooplankton feeding selectivity can be known.

2.2.2 Pigments as signature markers of phytoplankton

Some phytoplankton classes can be identified by unique pigments. These pigment markers are then used in HPLC analysis for qualifying and quantifying phytoplankton in water or zooplankton gut. Mapping of phytoplankton pigments for particular phytoplankton population started in the early of 1970s when researchers used thin-layer chromatography (TLC) for mapping of the profile of photosynthetic pigments for particular phytoplankton populations and biological processes (Table 2.1) (Jeffrey, 1968, 1974, 1976). With the development of automated HPLC methods for pigment separations in the 1980s, more pigments were separated (Repeta

& Gagosian, 1982; Mantoura & Llewellyn, 1983; Gieskes & Kraay, 1983*a*, 1983*b*, 1986*a*, 1986*b*; Kleppel & Pieper, 1984; Wright & Shearer, 1984; Bidigare *et al.*, 1985; Roy, 1987; Zapata *et al.*, 1987; Kleppel, 1988; Jeffrey, 1999; Wong, 2003). Signature pigments for phytoplankton populations were mapped by HPLC analysis due to the above advances. Recently, phytoplankton pigment markers can identify beyond family or class level (Jeffrey *et al.*, 1999; Wong, 2003). Table 2.2 shows the pigment markers for phytoplankton in division or class level in the late of 1990s.

Table 2.1. Definitive pigments used as markers of phytoplankton populations in the 1970s (Data from Ricketts, 1970; Jeffrey, 1974, 1976; Jeffrey et al., 1975; Wong, 2003).

Pigments	Phytoplankton groups or biological process
Chlorophyll-*a*	All phytoplankton groups except prochlorophytes
Chlorophyll c_1	Diatoms, chrysophytes
Chlorophyll c_2	Diatoms dinoflagellates
Fucoxanthin	Diatoms, chrysophytes
Diadinoxanthin	Diatoms, chrysophytes
Chlorophyll *b*	Chlorophytes
Neoxanthin	Chlorophytes
Peridinin	Dinoflagellates
Chlorophyllide *a*	Senescent diatoms (due to chlorophyllase action)
Pheophytin *a*	Usually present in traces on all chromatograms
High chlorophyll *c*:*a* ratios	Senescent phytoplankton or detrital material

Table 2.2 Summary of pigment markers for phytoplankton in division or class level in the late of 1990s (Data from Wright *et al.*, 1991; Jeffrey, 1997; Jeffrey & Vesk, 1997; Wong, 2003).

Phytoplankton division or class	Phytoplankton pigments
Cyanobacteria	Chlorophyll-*a*, β-carotene, zeaxanthin, allophycocyanins, phycocyanins and phycoerythrins
Prochlorophyta	Divinyl chlorophyll-*a* & *b*, α-carotene, β-carotene, zeaxanthin and a trace amount of phytylated chlorophyll *c*-like MgDVP
Rhodophyta	Chlorophyll-*a*, α-carotenezeaxanthin, allophycocyanins, phycoerythrins and a trace amount of phycocyanins
Cryptophyta	Chlorophyll-*a* and c_2, α-carotene, alloxanthin, phycocyanins, phycoerythrins and a trace amount of ε-carotene, lycoene, crocoxanthin & monadoxanthin
Chlorophyceae	Chlorophyll-*a* & *b*, β-carotene, lutein, neoxanthin, violaxanthin and a trace amount of α-carotene, γ-carotene & antheraxanthin
Prasinophyceae	Chlorophyll-*a* & *b*, phytylated chlorophyll *c*-like MgDVP, β-carotene, lutein, neoxanthin, prasinoxanthin c& violaxanthin and a trace amount of α-carotene & antheraxanthin
Euglenophyta	Chlorophyll-a & *b*, β-carotene, diadinoxanthin and a trace amount of antheraxanthin, diatoxanthin & neoxanthin
Eustigmatophyta	Chlorophyll-*a*, β-carotene, vaucheriaxanthin ester, violaxanthin and a trace amount of zeaxanthin
Bacillariophyta	Chlorophyll-*a*, *c1* & *c2*, diadinoxanthin, fucoxanthin and a trace amount of β-carotene & diatoxanthin
Dinophyta	Chlorophyll-*a* & *b*, diadinoxanthin, dinoxanthin, peridinin and a trace amount of β-carotene, diatoxanthin, P-457 + P-468, peridininol & pyrrhoaxanthin

Prymnesiophyceae	Chlorophyll-*a*, *c1*, *c2*, *c3*, α-carotene, 19'-butanoyloxyfucoxanthin, diadinoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin and a trace amount of phytylated chlorophyll c-like MgDVP, β-carotene & diatoxanthin
Chrysophyceae	Chlorophyll-*a*, *c2* & *c3*, 19'-butanoyloxyfucoxanthin, diadinoxanthin, fucoxanthin and a trace amount of β-carotene & diatoxanthin
Raphidophyceae	Chlorophyll-*a*, *c1* & *c2*, β-carotene, diadinoxanthin, fucoxanthin and a trace amount of diatoxanthin

2.2.3 Development of HPLC analysis of phytoplankton pigments

Since the 1980s, high performance liquid chromatography (HPLC) has been widely used in phytoplankton pigment analysis. Originally, this method was used in analysis of phytoplankton community and structure only, but not in analysis of zooplankton feeding selectivity. For the investigation of phytoplankton community, only qualitative data were obtained in the early period (Jeffrey & Hallegraeff, 1980, 1987; Hallegraeff & Jeffrey, 1984; Ridout & Morris, 1985;, Klein & Sournia, 1987). More recently, some investigators have used the HPLC method to quantify the abundances of different phytoplankton classes from pigment marker concentrations (Gieskes *et al.*, 1988; Everitt *et al.*, 1990; Letelier *et al.*, 1993). More recently, a new computer programme called CHEMTAX for calculating plankton class abundances from measured pigment concentrations and estimated class pigment composition was developed. Together with the availability of commercial pigment markers, the use of HPLC analysis of phytoplankton pigments on studies of marine ecosystems becomes more convenient.

The application of HPLC analysis of phytoplankton pigments on studying zooplankton feeding selectivity began in 1990s (Head & Harris, 1994; Quiblier-Llobéras *et al.*, 1996). Head and Harris (1994) used HPLC analysis of phytoplankton pigments to investigate feeding selectivity of zooplankton collected from an area of upwelling off the coast of Morocco in the 'JGOFS' study. Grazing experiments were carried out. The difference in phytoplankton abundances before and after grazing were compared to investigate the feeding selectivity behavior of the zooplankton. Quiblier-Llobéras *et al.* (1996) used HPLC analysis of phytoplankton pigments to investigate *in-situ* zooplankton feeding selectivity at Lake Pavin in the Central Massif of France. Comparisons between the proportions of phytoplankton pigments in water and zooplankton guts were done. Any differences indicated the occurrence

of zooplankton feeding selectivity. In recent years, HPLC analysis of phytoplankton pigments are widely used in studying zooplankton feeding selectivity (Swadling & Marcus, 1994; Buffan-dubau *et al.*, 1996; Kleppel *et al.*, 1996; Breton *et al.*, 1999; Meyer-Harms *et al.*, 1999; Gasparini *et al.*, 2000; Suzukim *et al.*, 2002).

2.2.4 Advantages of HPLC analysis of phytoplankton pigments

HPLC analysis of phytoplankton pigments is widely used in studying zooplankton feeding selectivity. This is due to the advantages of this method over other traditional methods. HPLC not only allows accurate quantitative analyses of chlorophylls (free from interference from degradation products), it also allows separation of up to 20 taxonomically useful carotenoids from mixed phytoplankton populations, with a total of over 40 pigments (chlorophylls, carotenoids and derivatives) being separated in a single run (Jeffrey *et al.*, 1999). More recently, commercial availability of pigment markers has made the taxonomic identification of phytoplankton and the identification of zooplankton gut contents more convenient. The HPLC method does not have the disadvantages of some of the traditional methods discussed earlier. Since different pigments are specific for individual phytoplankton taxa, the study of phytoplankton pigment diversity and concentration in the water column as well as in the zooplankton gut has become a useful tool when trying to describe the phytoplankton community in the water or the phytoplankton eaten by the zooplankton. *In situ* information is also available. Types of microalgae that had previously been missed in microscopic analysis of field samples through filtering loss or preservation damage could now be recognized by their pigment signatures (Gieskes & Kraay, 1983; Guillard *et al.*, 1985; Jeffrey et al., 1999).

2.2.5 Limitation of HPLC analysis of phytoplankton pigments

The HPLC method has some limitations. The sensitivity of this method is limited by pigment degradation, as in the case of the gut fluorescence method. Underestimation of grazing rates may occur. In addition, the rates of degradation appear to vary among pigments. Therefore, the proportions of different phytoplankton pigments in zooplankton gut obtained by HPLC might not reflect the actual situation. As a result, experiments evaluating the degradation efficiencies of phytoplankton pigments used for taxonomy were also carried out to verify the results of feeding selectivity of zooplankton determined by HPLC analysis of phytoplankton pigments.

2.3 Zooplankton feeding selectivity

2.3.1 Ecological importance of zooplankton feeding selectivity

Feeding selectivity of herbivorous zooplankton is important in affecting the structure and composition of the phytoplankton community. For example, Bergqust *et al.* (1985) showed zooplankton communities with different-sized individuals determined the composition of phytoplankton community. If the zooplankton only eats phytoplankton that is rare, the amount of energy transferred to the upper tropical levels will be small. The energy in the common phytoplankton species will go to the decomposers and the efficiency of trophic transfer will be low. In some cases, selective grazing of zooplankton will cause blooms of ungrazed phytoplankton species. On the other hand, zooplankton may be a control of phytoplankton bloom. Welschmeyer *et al.* (1991) found that copepods and salps could control diatom blooms in waters of the subarctic Pacific.

2.3.2 Factors affecting zooplankton feeding selectivity

Many studies showed that the quality of phytoplankton eaten by zooplankton may affect egg production of zooplankton (Stóttrup & Jensen, 1990; Jónastóttir, 1994; Jónastóttir *et al.*, 1995). Nutritional values of phytoplankton are one of the factors affecting zooplankton feeding selectivity. Dinoflagellates have a higher volume-specific organic content than diatoms under the same environment (Hitchcock, 1982). Therefore, they were recognized as an important part of the diet of calanoid copepods (Price et al., 1983; Gill and Harris, 1987; Kleppel et al., 1991; Kleppel, 1993; Meyer-Harms et al., 1999). *Phaeocystis* sp. has poor nutritional value (as polyunsaturated fatty acids and vitamin C) (Claustre *et al.*, 1990), and this may be one of the reasons why zooplankton select against *Phaeocystis* sp. In addition, under certain conditions, *Phaeocystis* sp. is gelatinous in some life-cycle phases. This is

the reason for why *Phaeocystis* sp. is not ingested by *Calanus helgoandicus* (Estep *et al.*, 1990; Bautista *et al.*, 1992). In addition to nutritional values, size of phytoplankton is an important factor affecting zooplankton feeding selectivity. Copepods often prefer larger phytoplankton (Frost, 1972; Allan *et al.*, 1977; Donaghay & Small, 1979, O'conners *et al.*, 1980; Ryther & Sanders, 1980; DeMott, 1989). If the phytoplankton available are of the same size, zooplankton select based on the protein or nitrogen content (Libourel Houde & Roman, 1987; Cowles et al., 1988), toxicity (Huntley *et al.*, 1986) and digestibility (DeMott, 1989) of the algae. Also, there are some evidences that larger copepods prefer larger food items, while smaller copepod or microzooplankton prefer smaller ones (Nival & Nival, 1976; Harris, 1982; Strom & Welschmeyer, 1991) or graze non-selectively (Allan *et al.*, 1977). In addition to the above, copepods will prefer living particles (Donaghay & Small, 1979). Phytoplankton abundance and motility was also the factors governing feeding selectivity of zooplankton (Allan *et al.*, 1977; Saiz, 1994; Saiz & Kiørboe, 1995; Kiørboe *et al.*, 1996; Kleppel *et al.*, 1996). Reason for the selection behavior resulted from phytoplankton abundance and motility may be the differences in the feeding mode of copepods. Saiz & Kiørboe (1995) and Kiørboe *et al.* (1996) found that *Acartia tonsa* have two feeding modes. One was suspension feeding mode and the other was ambush feeding mode. At low phytoplankton concentrations, clearance rate of *A. tonsa* was reduced. Copepods switched to ambush feeding. Motile preys became more important diets of copepods. These motile preys included dinoflagellates and cilitates.

2.3.3 Feeding selectivity of zooplankton studied in this study

2.3.3.1 *P. avirostirs*

The marine cladoceran *P. avirostirs* is one of the zooplankton studied in this research. Cladocerans are aquatic microcrustaceans. In freshwater, there are more than 600 recorded species (Schram, 1986). However, in the sea, there are only 8 true marine species (Onbé, 1977). *P. avirostirs* is one of the eight true marine species. It belongs to the family Sidiae. Information on the feeding habits of *P. avirostris* is limited. It is known that the main food of *P. avirostirs* included small diatoms, microflagellates and cyanobacteria (Gore, 1980; Lipej *et al.*, 1997; Paffenhöfer and Orcutt, 1986; Turner *et al.*, 1988; Kim *et al.*, 1989). *Penilia* has feeding appendages inside the carapace, and is considered to be a filter feeder. The setae are closely packed. It is known to feed on particles <15 μm (Gore, 1980; Paffenhöfer & Orcutt, 1986; Turner *et al.*, 1988; Kim *et al.*, 1989a; Lipej *et al.*, 1997). Therefore, it prefers to graze on nanoplankton and picoplankton (Gore, 1980; Paffenhöfer & Orcutt, 1986; Turner *et al.*, 1988; Kim *et al.*, 1989a; Lipej *et al.*, 1997) . It also grazes on heterotrophic microflagellates, autotrophic nanoplnaktonic flagellates, small diatoms, *Nitzschia*, cyanobacteria and other bacteria (Gore, 1980; Paffenhöfer & Orcutt, 1986; Turner *et al.*, 1988; Kim *et al.*, 1989a; Roff *et al.*, 1995; Lipej *et al.*, 1997) . Turner *et al.* (1998) showed that selective feeding by *P. avirostris* was not invariable, but rather fluctuated due to variations in grazer behaviour as well as composition of food assemblages. Grazing of cyanobacterium filaments for copepods and cladocerans was disproportionately low (Turner *et al.*, 1998). Filament length data suggest, however, that grazers also reduced lengths of many cyanobacterial filaments, possibly by biting off portions of filaments that were not totally consumed. Due to filament clipping, the impact of grazers upon cyanobacterial filaments is at some

considerably higher (but unresolved) level than is implied by the clearance and ingestion rates calculated from filament removal.

2.3.3.2 *Paracalanus* spp.

Calanoid copepods belonging to the genus *Paracalanus* are an important component of the planktonic copepod community in Tolo Harbour. Species of *Paracalanus* that can be found in Tolo Harbour include *P. aculeatus*, *P. crassirostris*, *P. gracilis* and *P. parvus* (Wong *et al.*, 1993). Wong *et al.* (1993) found that *Paracalanus* spp. occurred in large numbers in Tolo Harbor throughout the year. Therefore, *Paracalanus* spp. may influence the phytoplankton community and composition. As a result, *Paracalanus* spp. were also studied in this MPhil research. Previous studies showed that diatoms are the main food of *Paracalanus* spp. (Gasparini *et al.*, 2000). One of the *Paracalanus* spp., *P. parvus* is known to be capable of consuming small-sized phytoplankton. *P. parvus* can feed on 8 μm cells with 40-50% efficiency relative to large cells (Bartram, 1980; Fernandez *et al.*, 1993). However, if the sizes of *Paracalanus* spp. are larger, they will prefer larger phytoplankton (Paffenhöfer, 1984). A 34-hr investigation of the gut fluorescence revealed nocturnal feeding in *Paracalanus* spp. (Tsuda and Nemoto, 1988). Tang *et al.* also studied diel gut pigment rhythm of *Paracalanus parvus* and *P. crassirostris*. During the winter study between January 24 and 25, 1992, both *Paracalanus* species showed diel gut pigment rhythm.

2.3.3.3 *Pseudevadne tergestina*

Previous studies on the feeding selectivity of *P. tergestina* are limited. It feeds mainly on centric diatoms such as *Chaetoceros* spp. (Kim *et al.*, 1989a). *E. nordmanni* favors *Ceratium* (Nielsen, 1991; Rivier, 1998), while was often found to contain dinoflagellates (*Ceratium*, *Protoperidinium*) and tintinnids (Bainbridge, 1958; Rivier, 1998).

2.4 Pigment degradation in guts of zooplankton

2.4.1 Experimental design

Many investigators have studied pigment degradation in zooplankton guts (Lopez & Huntley, 1988; Penry & Frost, 1991; Head, 1992; Mayzaud & Razouls, 1992; Head & Harris, 1992, 1994; Suzanne *et al.*, 1998). In some zooplankton feeding experiments, initial and final concentrations of pigments or concentrations of pigments at different periods in water were measured (Head, 1992; Suzanne *et al.*, 1998). Fecal pellets were also collected and measured for the contents of pigments (Lopez & Huntley, 1988; Penry & Frost, 1991; Head, 1992; Mayzaud & Razouls, 1992; Head & Harris, 1992, 1994; Suzanne *et al.*, 1998). These data were then used to estimate the pigment degradation efficiency. The amount of pigments that have become undetectable were estimated. Various equations for calculating pigment degradation were used, but all calculations were based on the principle that the amount of pigments in water before incubation should be equal to that after incubation together with the pigments in fecal pellets. The decrease in amounts of pigments was due to pigment degradation.

2.4.2 Pigment degradation

The range of percentage of degraded chlorophyll-*a* varied widely from 10 to 90% (Head, 1988; Lopez et al., 1988; Penry & Frost, 1991; Head & Harris, 1992; Pasternak, 1994). Some researches showed that carotenoids were more stable than chlorophylls (Kleppel et al., 1988, 1991; Nelson, 1989). The presence of intact carotenoids in copepod guts or faecal pellets suggested that pigment analysis might provide a method of looking at in *situ* dietary selection (Kleppel et al. 1988, 1991). On the other hand, some researches found that chlorophylls were more stable than carotenoids (Head & Harris, 1992, 1994). Copepods metabolized the carotenoids

present in the algae, so that ingested fucoxanthin was completely absent from faecal pellets, and diadinoxanthin concentrations were very low (Head & Harris, 1992). Faecal pellets contained undetectably low levels of chl *c* and fucoxanthin. Ingested 19-hexanoyloxyfucoxanthin was also completely destroyed during grazing (Head & Harris, 1994). Therefore, the application of gut pigment analysis by HPLC to studies of feeding selectivity of zooplankton was criticized (Head & Harris, 1994). Nevertheless, pigment degradation in zooplankton guts could be affected by food concentration and previous feeding history (Penry & Frost, 1991; Head, 1992; Head & Harris, 1992). Additional factors including differences in zooplankton species or dietary composition and incubation conditions may also influence the degree of pigment destruction (Head 1992).

2.5 Tolo Harbour, Hong Kong

2.5.1 Site description

Tolo Harbour was chosen as the sampling site. Tolo Harbour is located in the northeast of Hong Kong (114°E, 22°N). Maximum depth is 24 m and total area is ~50 km^2. Formerly a rural area, the catchment of Tolo Harbour has been under intensive urbanization since the early 1970s. Increased sewage loading from the expanding population had caused a progressive increase in the occurrence of algal blooms, red tides and oxygen depletion in the past ten years (Wu, 1988; Wong *et al.*, 1992). The bottleneck topography of Tolo Harbour reduces tidal exchange and mixing of seawater by northeasterly winds providing environmental conditions that favour algal blooms (Watson & Watsons, 1971; Gordon, 1975; Chan & Hodgkiss, 1987; Hodgkiss & Ho, 1997). In the 1980s, there was a sharp increase in the number of red tide occurrences. The Government implemented the Tolo Harbour Action Plan (THAP) in 1986 to reduce nutrient loading into Tolo Harbour. Since the implementation of the THAP, the trend of water quality deterioration in Tolo Harbour has been effectively arrested. Monitoring data from 1986 to 2000 revealed a general decline in chlorophyll-*a* concentration & organic pollutants and significant decreases in nitrate nitrogen, total inorganic nitrogen and orthophosphate phosphorus nutrients. Water quality improvement has also led to a decrease in the number of red tides from 88 in 1988 to 20 in 2003.

2.5.2 Phytoplankton and zooplankton in Tolo Harbour

Phytoplankton monitoring in Tolo Harbour by the Hong Kong Environmental Protection Department found that about half of the total species consisted of diatoms and 39% consisted of dinoflagellates (HKEPD, 2001). The most abundant zooplankton in Tolo Harbour is copepod. However, four species of marine cladocerans can also be found in Tolo Harbour. The quantity of cladocerans is higher in the inner harbour. Therefore, Tolo Harbour is a good area to study the grazing behaviour of cladocerans. *Paracalanus* spp. are the dominant copepods in Tolo Harbour.

CHAPTER 3 MATERIALS AND METHODS

3.1 Field sampling

The research was mainly divided into two parts as shown in Figure 3.1. The first part was a study of seasonal pattern in zooplankton feeding selectivity in the Tolo Harbour. The second part was a series of laboratory feeding experiments to estimate pigment degradations and clearance rates of zooplankton. Both of these two parts consisted of field sampling followed by laboratory analysis or experiments.

3.1.1 Study of seasonal patterns in zooplankton feeding selectivity

Sampling was carried out at two stations in the inner part of Tolo Harbour (Station I: 22° 25.103' N; 114°13.133' E; Station II: 22°26.176' N; 114°12.87' E) (Figure 3.2). The depth of the stations were from 6m to 10m depedig on the tides on the sampling days. Phytoplankton and zooplankton samples were collected in February, March, April, May, June, August, October, November & December 2003 and January 2004. Temperatures were measured during each sampling.

3.1.1.1 Collection of phytoplankton and zooplankton for pigment analysis

On each sampling trip, duplicate seawater samples were collected at the surface of the sea at each sampling station with a 2-L opaque bottles for HPLC analysis of phytoplankton pigments and fluorometric analysis of chlorophyll-*a*. The water samples were kept in a dark icebox at ~4°C immediately after sampling. They were returned to the laboratory immediately and were stored in a refrigerator at 4°C for no more than 1 day before processing. Water samples intended for HPLC pigment analysis were passed through Whatman GF/C filters and stored at -20°C until pigment extraction. Water samples intended for fluorometric analysis of chlorophyll-*a* were filtered with Millipore™ membrane filters (0.45 μm HA). The

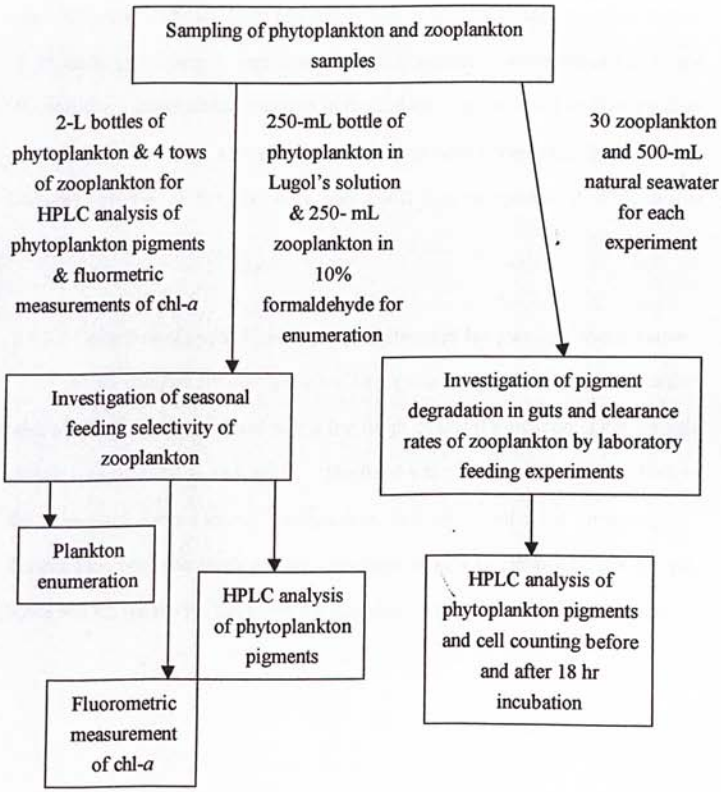


Figure 3.1 Outline of experimental design.

filters were stored at -20°C in darkness until pigment extraction. The detailed experimental procedures are illustrated in section 3.2.

Immediately after collection of water samples, zooplankton samples were collected using a plankton net of 0.5-m diameter and 125-μm mesh. The plankton net was hauled vertically from near the bottom to surface at each sampling station. Four hauls were made at each station for gut pigment analysis using HPLC and fluorometer. Zooplankton collected in the cod end was collected on 200 μm mesh nettings. Mesh nettings with zooplankton samples were immediately fixed in liquid nitrogen, returned to the laboratory, and stored in a refrigerator at –80°C before analysis.

3.1.1.2 Collection of phytoplankton and zooplankton for plankton enumeration

Water samples for phytoplankton enumeration were collected at the surface with a 250 mL bottle and fixed with a few drops of Lugol's solution. Only a single sample was collected at each station. The fixed water samples were carried back to the laboratory for taxonomic identification and enumeration of phytoplankton. Zooplankton from one single net haul was fixed in 10% formaldehyde in a 250 mL bottle and returned to the laboratory for taxonomic identification and enumeration.

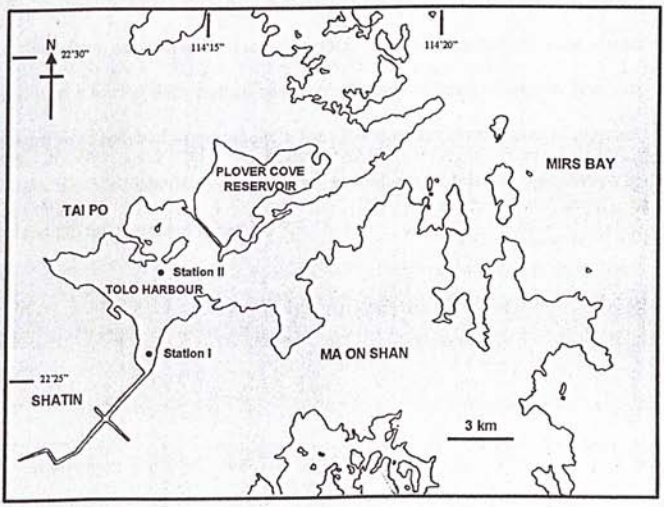


Figure 3.2 Sampling stations in Tolo Harbour

3.1.2 Collection of phytoplankton and zooplankton for laboratory feeding experiments

Penilia avirostris and *Paracalanus* spp. were collected at Station I on 3 and 30 July 2003, 4 August 2003, 2 October 2003, and 6 November 2003 for laboratory study of pigment degradation in zooplankton guts. Natural surface seawater was collected in two 2-L opaque bottles on each sampling trip. Zooplankton was collected by making single haul with a plankton net of 0.5-m diameter and with a 125-μm mesh from near the bottom to the surface. The live zooplankton were placed temporarily in a bucket with surface seawater. Freshly collected seawater and live zooplankton were returned immediately to the laboratory for experiments of pigment degradation. Detailed laboratory experimental procedures for pigment degradation in zooplankton gut are presented in section 3.3.

3.2 Laboratory experiments and data analysis

3.2.1 Study of seasonal patterns in zooplankton feeding selectivity

3.2.1.1 HPLC of phytoplankton pigments

Surface seawater samples were filtered through 47 mm diameter Whatman GF/C filters immediately or within one day after sampling to collect the phytoplankton. Five hundreds mL of surface seawater was filtered onto one filter paper (400 mL for laboratory grazing experiments). The filter papers were stored in darkness at –80°C until pigment extraction and analysis. Pigment extraction and analysis were carried out within one month after sampling to minimize any changes of pigment quality and quantity during storage (Wright et al., 1991). During pigment extraction, filters were cut into small pieces and ground with aluminium rod in 1.8 mL of chilled 100% acetone (HPLC grade; Sigma) in centrifuge tubes wrapped with aluminium foils. The ground filters were then sonicated for 30 seconds and extracted overnight at –20°C. After one night, milli-Q water was added to produce a final acetone concentration of 90%. The samples were further sonicated for 30 seconds and then shacked using a vortex. The samples were centrifuged for 6 minutes at 4,800 rpm. Supernatants were collected using disposable syringes and passed through Nalgene™ syringe fitted with membrane filters (0.2 µm pore size, 13 mm diameter). Sample extracts were analyzed by HPLC to find out the pigment composition in seawater samples. Analysis by HPLC after extraction was carried out within 48 hours to minimize pigment losses through precipitation and adsorption (Wright *et al.*, 1991; Mantoura *et al.*, 1997; Wong, 2003). All the above procedures were carried out under dim illumination. Two replicates were used for each station on each sampling day.

Mesh nettings with zooplankton were stored at –80°C immediately after returning to the laboratory until gut pigment extraction. For gut pigment extraction,

zooplankton samples were defrosted at 4°C in a small dish containing filtered seawater. *P. avirostris* and *Paracalanus* spp. were sorted under a dissection microscope. Sorted animals were put onto 0.45 µm Millipore nitrocellulose filters. Each filter contained up to 500 individuals of a single species. The sorted zooplankton was stored in an icebox at 4°C temporarily during sorting to minimize pigment degradation. After sorting enough individuals were sorted, the filter papers with zooplankton were extracted in 0.45 mL of 100% HPLC grade acetone. The extracted samples were then sonicated for 30 seconds. Zooplankton gut pigments were extracted overnight, then 0.05 mL of Milli-Q water was added to the sample to make a final acetone concentration of 90%. After these procedures, the samples were analyzed for phytoplankton pigments. Only one sample was examined per sampling station.

The procedures for HPLC pigment analysis were similar to those described by Wong (2003). A Hewlett Packard HP 1100 series HPLC was used. It composed of a quaternary pump with online degasser, an injector with injection valve of 20-25 µl sample loop, a multi-wavelength UV-VIS detector (190-950 nm), a multi-signal fluorescence detector and an agilent eclipse XDB-C18 polymeric reversed phase column (4.6 mm ID x 25 cm, 5 µm particle size) with a flow rate of 1.0 mL min^{-1}. The mobile phase setups were as follow:

Solvent A = 80:20 methanol : 0.5 M ammonium acetate at pH 7.2

Solvent B = 90:10 v/v acetonitrile : milli-Q water

Solvent C = pure ethyl acetate

Methanol, acetonitrile and ethyl acetate were HPLC grade. Ammonium acetate was analytical grade. The solvents were sonicated to remove the gases that might block the membrane filter in the HPLC. The method (schedule of solvents flowing through

the column during sample run) used by Wright *et al.* (1991) was followed with minor modification.

0 min: 100% solvent A

4 min: 100% solvent B

18 min: 20% solvent B, 80% solvent C

21 min: 100% solvent B

23.5 min: 100% solvent A

29 min: 100% solvent A (end)

Detection of all pigment by UV/VIS detector was at 436 nm with 385 nm as the reference wavelength. A fluorescence detector (excitation: 425 nm, emission: 660 nm) was used for verification of the fluorescent pigments. The Hewlett Packard HPLC ChemStation integrator-processor was used to process the results.

Phytoplankton pigments (chlorophyll *c3*, peridinin, fucoxanthin, 19-hexa-fucoxanthin, alloxanthin, lutein, chlorophyll *b* and chlorophyll-*a*) were identified by comparing with the retention times with those of authentic standards from the International Agency for ^{14}C Determination, Hørsholm, Denmark. Pigment concentrations were calculated by working out the equations for calibration curves of different pigments. At least five pigment concentrations were diluted from the pigment standard for calibration.

3.2.1.2 Fluorometric measurement of chlorophyll-*a*

Seawater and zooplankton samples collected for investigation of seasonal feeding selectivity of zooplankton were also analyzed by fluorometer. The concentrations of chlorophyll-*a* and its derived pigments in phytoplankton and zooplankton samples measured by fluorometer were compared with those obtained

from HPLC. The aim was to use the fluorometric method to verify results obtained by the HPLC pigment analysis method.

For seawater samples, 10 mL subsamples were filtered through 0.45 μ m Millipore nitrocellulose filters. For zooplankton samples, 30 individuals for each species were sorted under a dissecting microscope onto a 0.45 μ m Millipore nitrocellulose filter. Five mL 90% acetone (analytical grade) was added to each filter in 10 mL glass tubes. The openings of the glass tubes were wrapped with parafilms to minimize evaporation of the solvents. All the tubes were wrapped with aluminium foil to minimize photo-degradation of the pigments. Two replicates were done for each field sample. The samples were extracted overnight at 4°C in a dark refrigerator. After extraction, the samples were taken out from the refrigerator and allowed to stand for some times at room temperature before measurement with the fluorometer. Fluorescences of the sample extracts before and after acidification with 60 μ l of 10% HCl were measured by a fluorometer. The concentration of chlorophyll-*a* in the samples were calculated using the equation of Parson *et al.* (1984):

Chlorophyll-*a* concentration (ng mL^{-1}) = $K(R_b-R_a)v/V$ (seawater sample)

Chlorophyll-*a* concentration (ng mL^{-1}) = $K(R_b-R_a)v/N$ (zooplankton sample)

where K is the machine calibration constant, R_b and R_a are the fluorescence reading before and after acidification, v is the volume of acetone extract, V is the volume of seawater sample filtered and N is the number of zooplankton respectively. No correction of background fluorescence was done (Wong *et al.*, 1992).

3.2.1.3 Plankton identification and enumeration

Phytoplankton samples preserved in Lugol's solution were concentrated to 10 mL by settling the samples for more than a week in 100 mL measuring cylinders.

For enumeration of phytoplankton, two 1 mL aliquots of concentrated subsamples were transferred to a counting chamber. Total phytoplankton cell density and densities of dinoflagellates and diatoms were determined under an inverted microscope. For enumeration of zooplankton in seawater, 10 mL subsamples were taken from the 250 mL samples. Two 2.5 mL subsamples were then further taken and counted under a dissection microscope. A third 2.5 mL subsample was counted if there were more than 20% difference between the original two subsamples. The one subsample with the greatest deviation was then discarded. Densities of *Paracalanus* spp., other copepods, *P. avirostris*, *Pseudevadne tergestina* and total zooplankton were recorded. *Paracalanus* spp. were not identified to the species level. However, there were only 4 species (*P. aculeatus*, *P. crassirostris*, *P. gracilis* and *P. parvus*) in Tolo Harbour (Wong *et al.*, 1993). From the findings by Wong *et al.* (1993), the dominant *Paracalanus* spp. were *P. crassirostris* in January, August and November and *P. aculeatus* in May.

3.2.2 Laboratory feeding experiments - for investigation of pigment degradation in zooplankton gut

Live zooplankton and natural seawater were collected at MSL station and immediately returned to the laboratory for grazing experiments. In the laboratory, *P. avirostris* and *Paracalanus* spp. were sorted immediately under a dissection microscope. Grazing experiments were conducted in 500 mL opaque bottles. Each bottle contained 30 *P. avirostris* or 30 *Paracalanus* spp. Each type of zooplankton (*P. avirostris* and *Paracalanus* spp.) was fed two types of food media and two replicates were done for each experiment. One culture medium consisted of natural seawater from the field. This seawater was filtered through 150 μm mesh net to remove the large particles. The other culture medium consisted of filtered natural

seawater enriched with laboratory cultured chrysophytes. All together, there were eight experiment bottles and two control bottles. There were no zooplankton in the control bottles. Before the incubation, 30 *P. avirostris* and 30 *Paracalanus* spp. were frozen with liquid nitrogen and then measured for gut pigment content by fluorometer. In addition, the seawater used for the experiment was analyzed by HPLC for phytoplankton pigments. The phytoplankton community in the seawater before the experiment was also analyzed by cell counting. The ten bottles were incubated for 18 h in a large outdoor fish tank with circulating seawater. After the incubation, the bottles were removed from the fish tank. The zooplankton was collected on a mesh netting and used for measurement of gut pigment content using fluorometer. For the remaining seawater, 400 mL of seawater was filtered onto a filter paper. This was then used for HPLC analysis of phytoplankton pigments. The remaining seawater sample was also analyzed by cell counting method using inverted microscope.

The equation of Pasternak (1994) was used for calculating the degree of pigment degradation:

$$D^* = (\Delta - S_f)/\Delta \ \%$$

where D^* was the degradation efficiency, Δ was the amount of pigment ingested in ng, and S_f was pigment level in fecal pellets in ng. The equation was based on the assumption that phytoplankton pigment contents in zooplankton guts before and after the experiment were about the same. This assumption was verified by comparing the fluorometrically derived chlorophyll-*a* contents in zooplankton guts before and after incubation. At the beginning of the experiment, total phytoplankton pigments in the bottle consisted of phytoplankton pigments in the water (C_0) and phytoplankton pigments in zooplankton guts (G_0). After the experiment, total phytoplankton pigments in the bottle consisted phytoplankton pigments remaining in the water (C_t),

phytoplankton pigments in zooplankton guts (G_{18}) and phytoplankton pigments in zooplankton feces (S_f). Therefore, phytoplankton pigments degraded by zooplankton should be equal to total phytoplankton pigments in the bottle before grazing by zooplankton minus total phytoplankton pigments in the bottle after grazing by zooplankton:

$$\text{Pigment degraded} = (C_0 + G_0) - (C_r + G_{18} + S_f)$$

Since G_0 and G_{18} were assumed to be about the same when gut content was in equilibrum, the equation can be reduced to:

$$\text{Pigment degraded} = C_0 - C_r - S_f$$

$$= \Delta - S_f$$

Because fecal materials of *P. avirostris* dissolved in the seawater after egestion, total concentration of phytoplankton pigments in seawater after incubation (C_{18}) could be expressed as ($C_r + S_f$), and

$$C_0 - C_{18} = \Delta - S_f$$

For *Paracalanus* spp., the calculation was similar as above. During calculation, Δ was estimated by finding out C_r and, C_r was detemined using the data from cell counting.

$$C_r = C_0 \ (K_r / K_0)$$

where K_0 was the phytoplankton cell concentration in the bottle at time 0 (cell mL^{-1}) and Kr was phytoplankton cell concentration in the bottle after grazing by zooplankton for 18 hr (cell mL^{-1}). All the data were corrected by taking into account changes in phytoplankton concentrations in the control bottles.

In addition to pigment degradation efficiencies, clearance rates of *P. avirostris* and *Paracalanus* spp. in the feeding experiments were calculated using the equations of Frost (1972):

$$C_2 = C_1 e^{k(t_2 - t_1)}$$

$$C_2^* = C_1^* e^{(k-g)(t_2-t_1)} \qquad F = Vg/N,$$

where C_1 and C_2 are cell or pigment concentrations (cells/mL) in the control bottle at t_1 and t_2. k is the growth constant for algal growth. C_1^* and C_2^* are cell or pigment concentrations in a bottle with grazers at time t_1 and t_2. g is the grazing coefficient. F is the clearance rate (Harvey, 1937). V is the volume (mL) of the bottle and N is the number of zooplankton in the bottle. Total phytoplankton cell concentrations obtained by cell counting using inverted microscope and different phytoplankton pigment marker concentrations obtained by HPLC analysis was used for calculation. The results were compared. As there were no significant difference between C_1 and C_2, k is zero during calculation. V is 500mL. N is 30 individuals.

CHAPTER 4 RESULTS

4.1 Information on Tolo Harbour

4.1.1 Temperature and seawater salinity in Tolo Harbour

The climate in Hong Kong is hot in summer and warm in winter. The temperature difference between daytime and nighttime is not large. Figure 4.1 shows the surface water temperatures in Tolo Harbour at the two sampling stations during the study period in 2003 and 2004. The surface seawater temperatures ranged from 18.5°C in January to 31.8°C in August. Therefore, the research was carried out at different temperatures. Grazing rates and pigment degradation for zooplankton might vary at different temperatures. Surface salinity in Tolo Harbour was about 34‰. There were no detectable changes in surface salinity in Tolo Harbour at both stations throughout the study period.

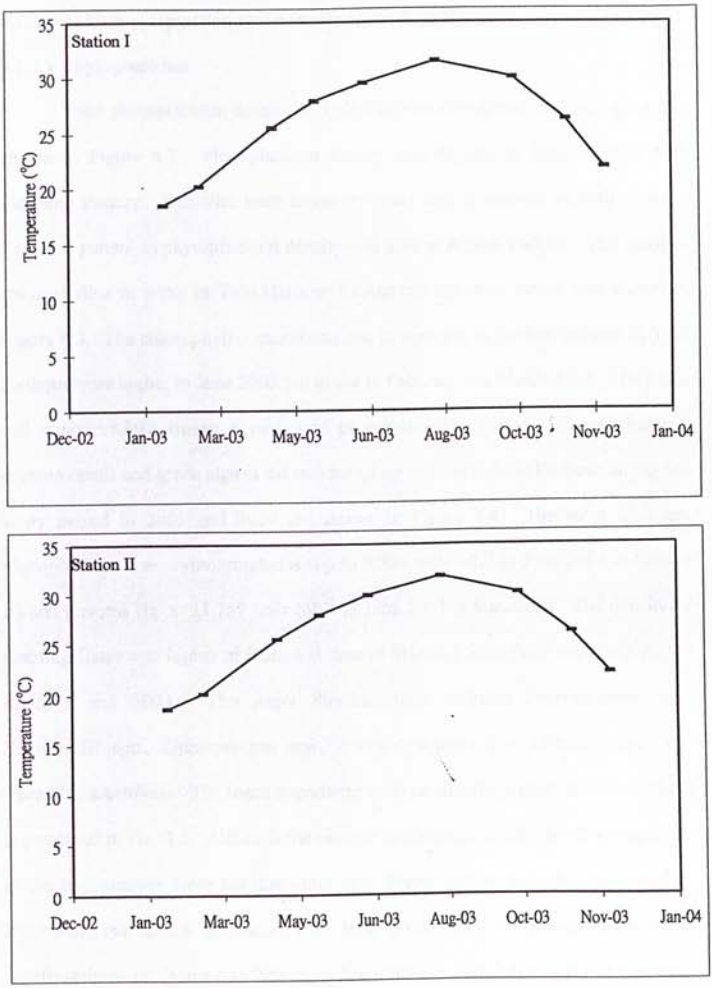
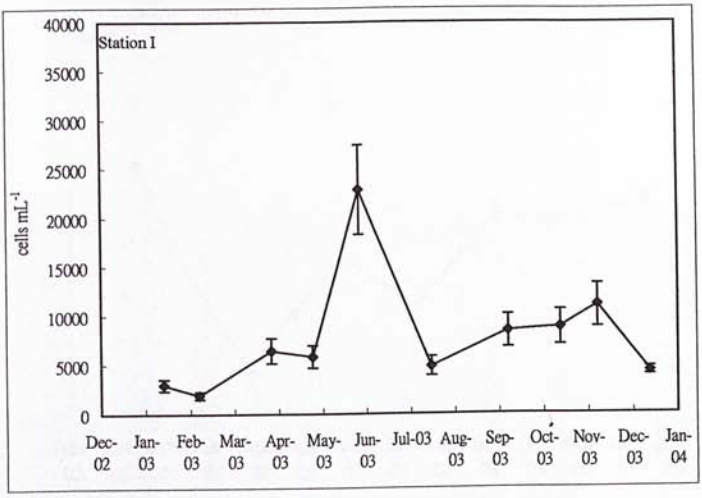


Figure 4.1 Surface water temperature (mean ± range) at two stations in Tolo Harbour during the study period in 2003 and 2004.

4.1.2 Plankton composition and community in Tolo Harbour

4.1.2.1 Phytoplankton

Total phytoplankton density in Tolo Harbour throughout the study period is shown in Figure 4.2. Phytoplankton density was highest in June 2003 at both sampling stations. Densities were lower in winter than in summer at both stations. Seasonal pattern in phytoplankton density was similar at both stations. The trend of chlorophyll-*a* in water in Tolo Harbour throughout the study period was shown in Figure 4.3. The chlorophyll-*a* concentrations in seawater at the two stations in Tolo Harbour were higher in June 2003 but lower in Feburary and March 2003. The mean cell concentrations (mean $\pm$ range) of prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green alga at the two sampling stations in Tolo Harbour during the study period in 2003 and 2004 are shown in Figure 4.4. The most abundant phytoplankton was prymnesiophytes (up to 9,586 cells mL^{-1} in June 2003 at Station II) and diatoms (up to 11,757 cells mL^{-1} in June 2003 at Station II). The density of dinoflagellates was higher at Station II than at Station I throughout the study period in 2003 and 2004. The major dinoflagellates included *Prorocentrum* spp., *Scrippsiella* spp., *Gymnodinium* spp., *Protoperidinium* spp., *Ceratium* spp. and *Noctiluca scintillans*. The mean abundance of these dinoflagellates in Tolo Harbour is presented in Fig. 4.5. Although the relative abundances of different dinoflagellates at the two stations were not the same (e.g. *Prorocentrum* at 1,180 cells mL^{-1} at Station II, but absent at Station I in June 2003), there were high densities of dinoflagellates in January to March at both stations. In other parts of the year, dinoflagellate densities were low not abundant at both stations. The most dominant dinoflagellates were *Prorocentrum* spp. and *Scrippsiella trochoidea*, which together accounted for 30 to over 90% of the dinoflagellate community in Tolo Harbour at the sampling stations during the study period in 2003 and 2004. During the study



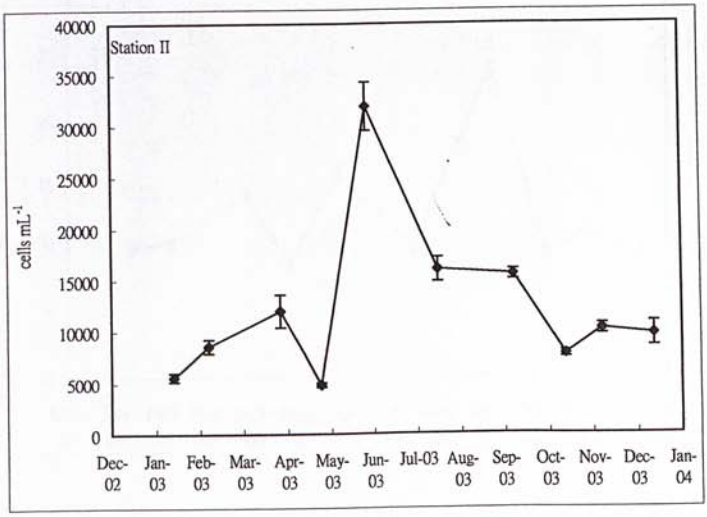


Figure 4.2 Total phytoplankton density (mean ± range) at the two stations in Tolo Harbour during the study period in 2003 and 2004.

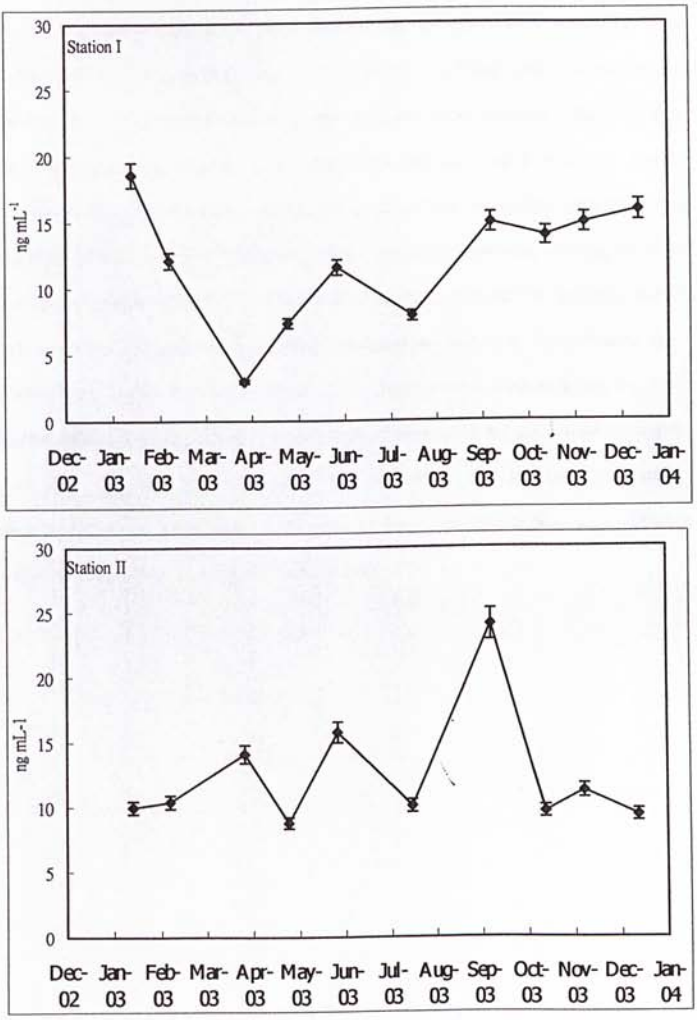


Figure 4.3 Temporal changes in chlorophyll-*a* content (ng mL^{-1}) (mean $\pm$ range) in water samples at two sampling stations in Tolo Harbour during the study period in 2003 and 2004.

period, the highest densities of *Prorocentrum* spp. and *Scrippsiella trochoidea* were 1,180 cells mL^{-1}in June 2003 and 2,942 cells mL^{-1} in Mach 2003, respectively at Station II. For diatoms, major groups included Coscinodicales, Biddulphiales, Rhizosoleniales, Naviculales, Diatomales, Phaeodactylales and Surirellales. Figure 4.6 shows the mean abundance of different groups of diatoms in Tolo Harbour during the study period. The most abundant diatoms were Coscinodicales. During the study period, the highest density of Coscinodicales was 8600 cells mL^{-1}in June at Station I. The common Coscinodicales included *Skeletonema costatum*, *Thalassiosira* spp., *Leptocylindrus* spp. and *Coscinodiscus* spp. Other common diatoms during the study period included *Thalassionema nitzschioides*, *Pseudonitzschia* spp., *Asterionellopsis glacialis*. Diatom density was highest in June 2003 at both stations. The highest diatom density recorded was 11,757 cells mL^{-1} in June 2003 at Station II. Diatom densities were lower in winter than in summer.

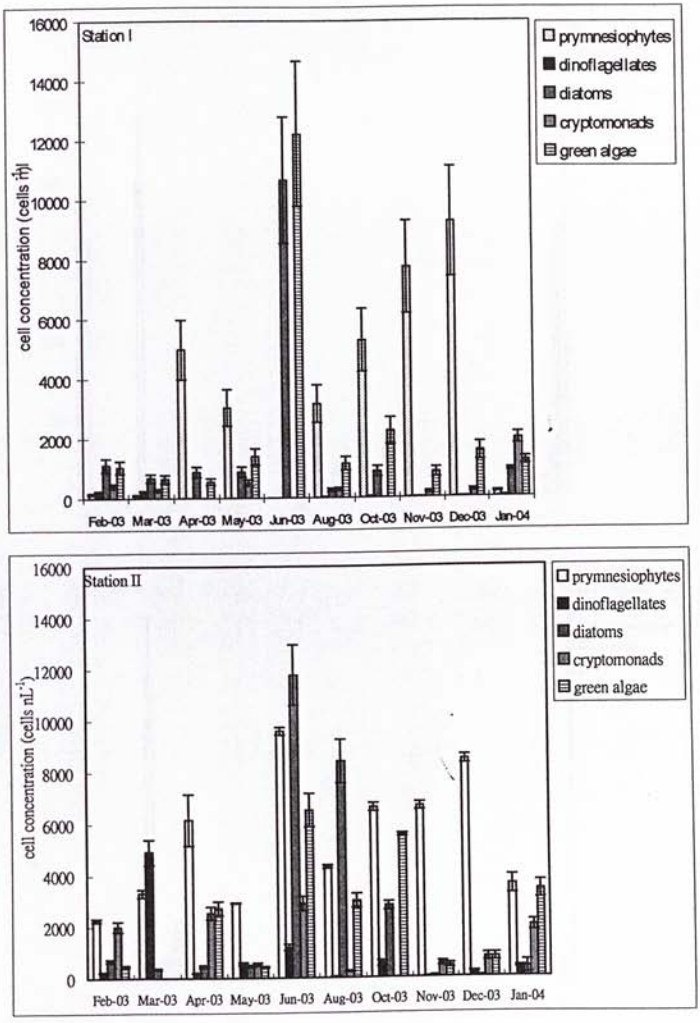


Figure 4.4 Mean density (mean ± range) of different phytoplankton groups at two sampling stations in Tolo Harbour during the study period in 2003 and 2004.

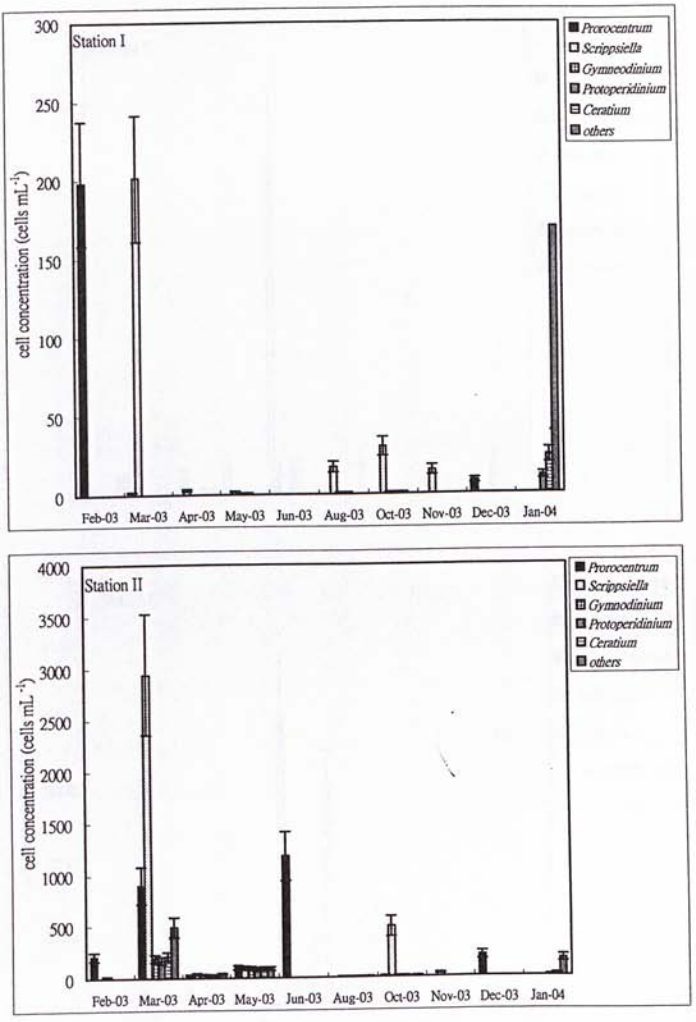


Figure 4.5 Mean density of major dinoflagellates (mean ± range) in Tolo Harbour during the study period in 2003 and 2004 at two stations.

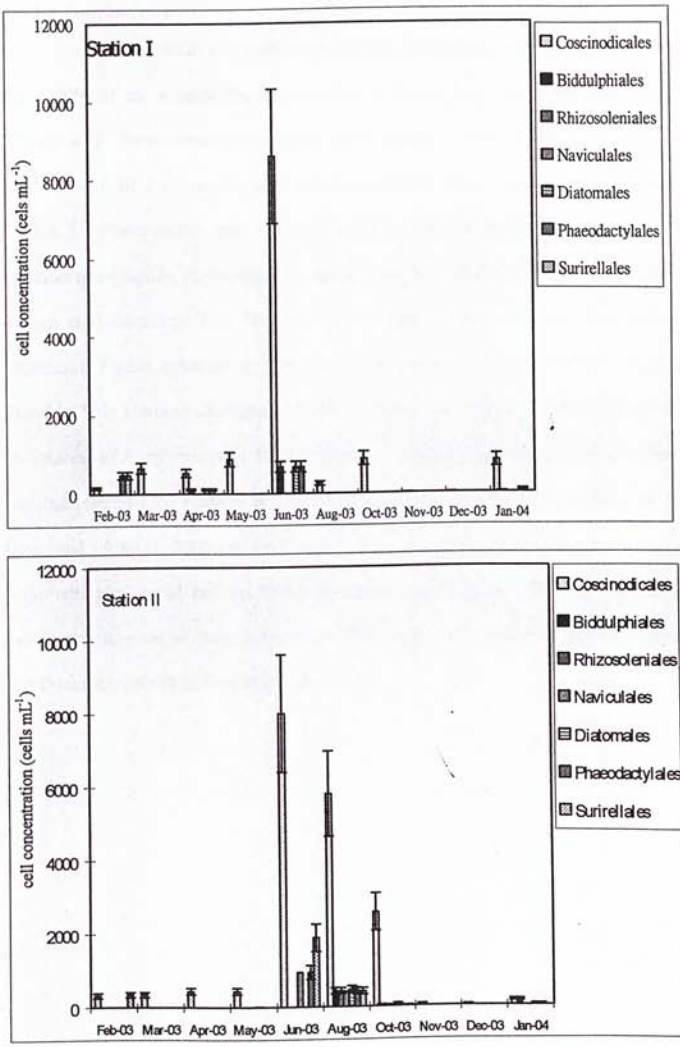


Figure 4.6 Mean abundance of major diatoms (mean ± range) in Tolo Harbour during the study period in 2003 and 2004 at two stations.

4.1.2.2 Zooplankton

In Tolo Harbour, copepods were the most dominant zooplankton, accounting for > 90% of the zooplankton composition at most times during the study period (Figure 4.7). *Paracalanus* spp. was the most abundant copepod. For example, more than 90% of the total zooplankton was *Paracalanus* spp. in August and October at Station I. *Paracalanus* spp. occurred in Tolo Harbour throughout the year. In addition to copepods, cladocerans could be found in Tolo Harbour. There were four marine cladocerans in Tolo Harbour. They were *Penilia avirostris*, *Pseudevadne tergestina*, *Podon schmackeri*, and *Podon polyphemoides*. *P. avirostris* could be found in Tolo Harbour throughour the year. There was no specific seasonal trend of abundance of *P. avirostris* in Tolo Harbour. However, the density of *P. avirostris* was the highest (362 number m^{-3}) in March 2003 and the lowest (2 number m^{-3}) in June and August 2003 at Station I. *P. tergestina*, *P. schmackeri* and *P. polyphemoides* could not be found in throughout the year Tolo Harbour as *P. avirsotris*. Blooms of these three cladocerans occurred in some months (November and December 2003) in Tolo Harbour.

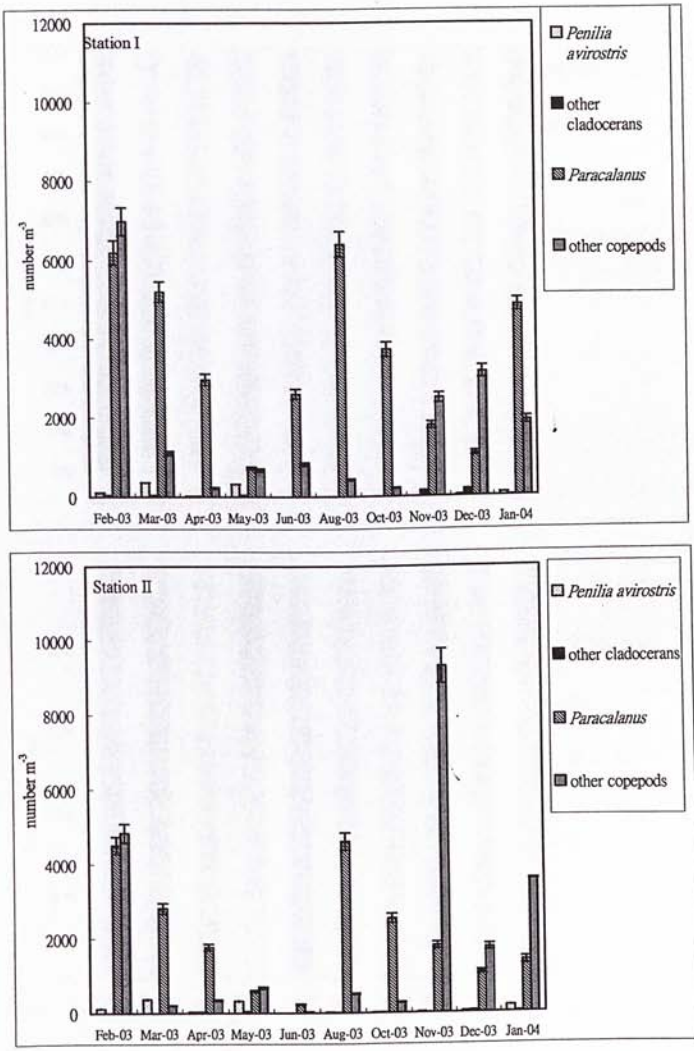


Figure 4.7 Mean density of different zooplankton groups (mean ± range) in Tolo Harbour during the study period in 2003 and 2004 at two stations.

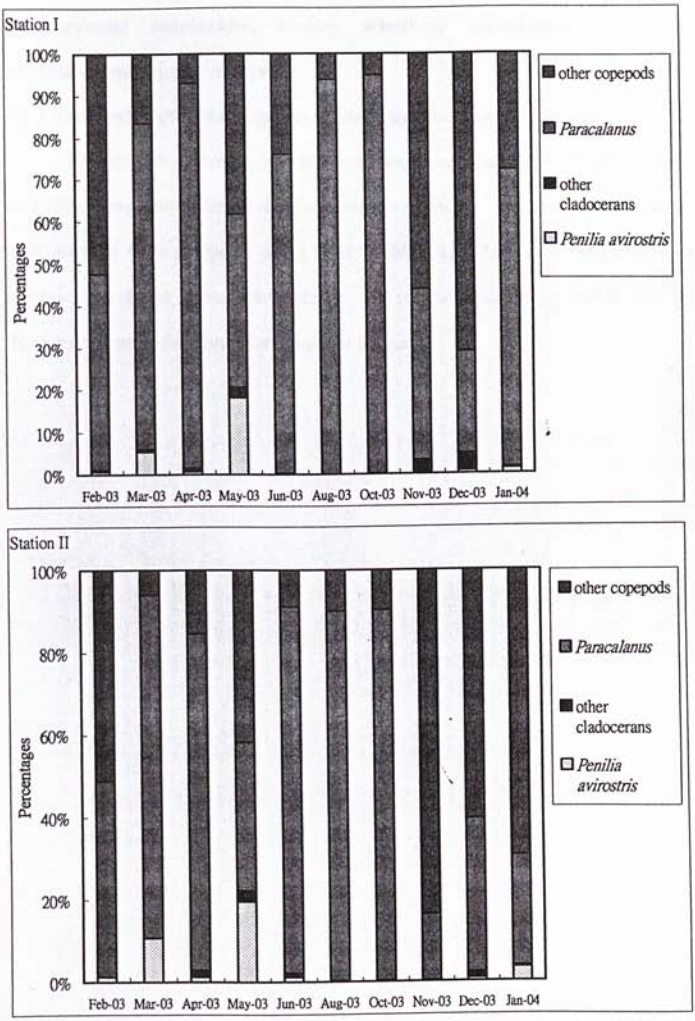


Figure 4.8 Relative proportion of different zooplankton groups (expressed as percentage of the sum of all the zooplankton) in Tolo Harbour during the study period in 2003 and 2004 at the two stations.

4.2 Seasonal zooplankton feeding selectivity investigated by HPLC phytoplankton pigment analysis

4.2.1 Verification of HPLC pigment analysis by fluorometric analysis

Chlorophyll-*a* contents in water and zooplankton samples were analyzed by both HPLC pigment analysis and fluorometric methods. The results obtained by both methods were compared using t-test (Table 4.1). Results showed that there were no significant differences between the results obtained by HPLC and by fluorometer except for *Paracalanus* spp. at Station II.

Table 4.1 Chlorophyll-*a* concentrations in phytoplankton and zooplankton samples taken from two sampling stations in Tolo Harbour. Results obtained from HPLC and fluorometric methods are compared using t-test. (ns = no significant difference at $P < 0.5$)

Samples	Stations	Methods	Chl-*a* (ng mL^{-1}) (mean ± SD)	*n*	*t*	*P*
Phytoplankton	Station I	HPLC	12.00 ± 4.70	10	1.45	ns
		Fluormetric	11.69 ± 4.75	10		
	Station II	HPLC	12.30 ± 4.67	10	0.69	ns
		Fluormetric	12.17 ± 4.94	10		
P. avirostris	Station I	HPLC	0.09 ± 0.04	10	1.31	ns
		Fluormetric	0.08 ± 0.03	10		
	Station II	HPLC	0.12 ± 0.09	10	1.75	ns
		Fluormetric	0.11 ± 0.09	10		
Paracalanus	Station I	HPLC	0.11 ± 0.14	10	1.27	ns
		Fluormetric	0.10 ± 014	10		
	Station II	HPLC	0.14 ± 0.13	10	1.10	ns
		Fluormetric	0.12 ± 0.14	10		

4.2.2 Correlations between phytoplankton cell densities and piment concentrations in water samples

Temporal changes in the densities of different phytoplankton groups were compared with changes in the concentrations of the corresponding phytoplankton pigment markers in seawater samples (Figures 4.9A – 4.18A). Chlorophyll-*c3*, peridinin, fucoxanthin, alloxanthin and lutein were used as the pigment markers of prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green alga, respectively. The trends of temporal changes in most phytoplankton group densities were similar to the trends of the corresponding phytoplankton pigment marker concentrations. There were significant linear correlations between the five phytoplankton groups and the corresponding pigment markers with one exception (critical value = 0.632, $R^2 > 0.632$ means significant linear correlation.). No significant linear correlation was found between dinoflagellates and peridinin at Station I (Figure 4.11). However, high significant linear correlation between dinoflagellates and peridinin was recorded at Station II. Therefore, peridinin was still used as the marker for dinoflagellates in my study. Chlorophyll-*c3*, peridinin, fucoxanthin, alloxanthin and lutein would be used as the pigment markers of prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green alga, respectively in later sections of this thesis. Linear regressions were used to determine the correlations between chlorophyll-*a* concentrations and the densities of total phytoplankton (Figures 4.19B & 4.20B), prymnesiophytes (Figure 4.21), dinoflagellates (Figure 4.22), diatoms (Figure 4.23), cryptomonads (Figure 4.24) and green algae (Figure 4.25). The results showed that chlorophyll-*a* concentrations in seawater did not have a significant linear correlation with the denisity of any of the above phytoplankton categories.

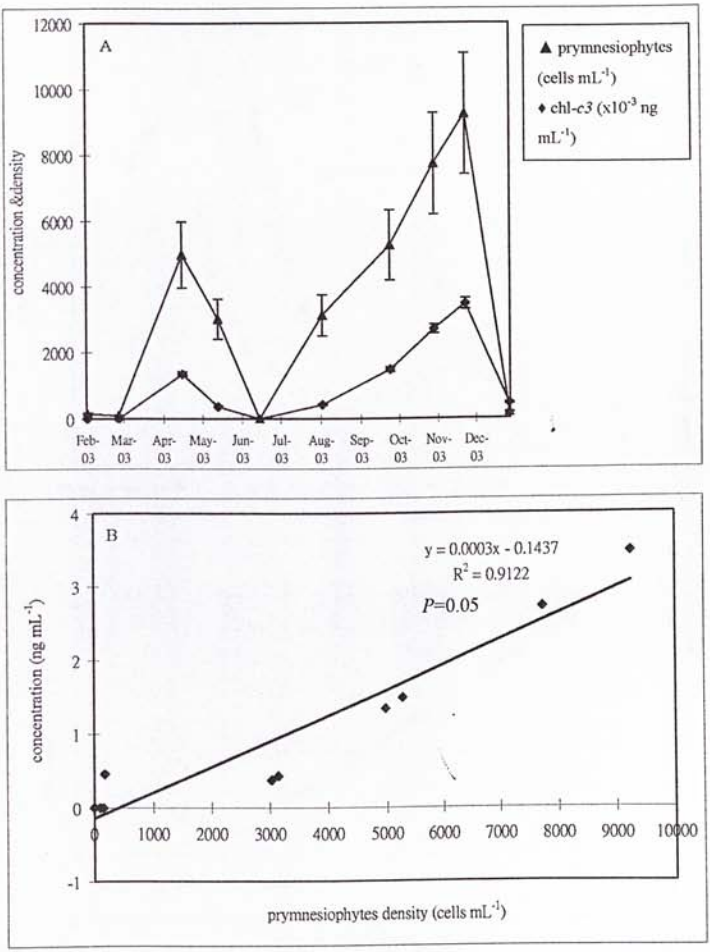
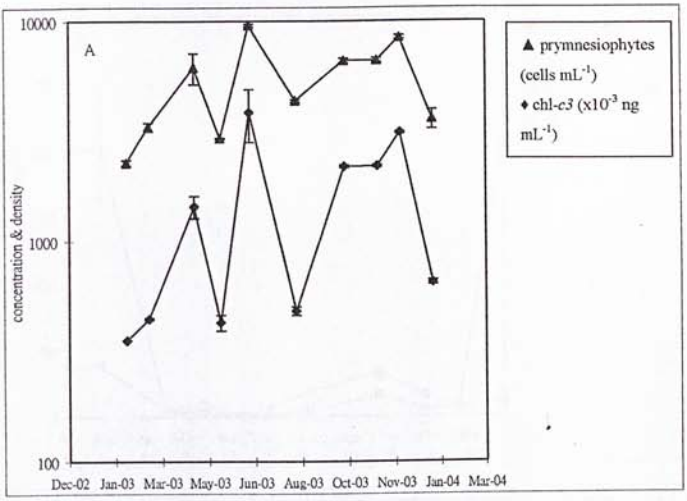


Figure 4.9 A. Temporal changes in chlorophyll-*c3* concentration ($x10^{-3}$ ng mL^{-1}) (mean ± range) and density of prymnesiophytes (cells mL^{-1}) (mean ± range) in water samples at Station I in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between chlorophyll-*c3* concentration and prymnesiophyte density at Station I.



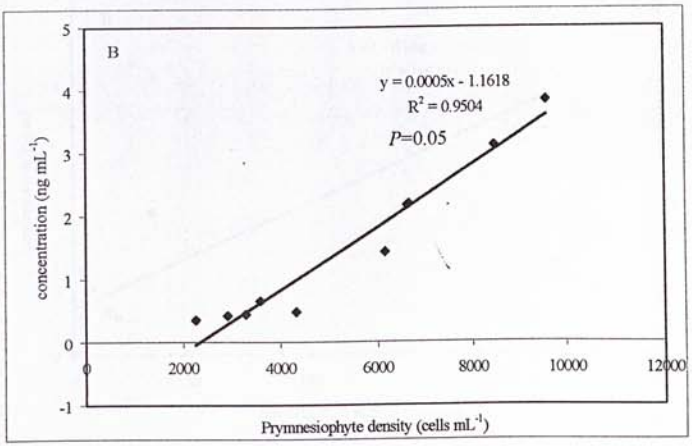


Figure 4.10 A. Temporal changes in chlorophyll-*c3* content ($x10^{-3}$ ng mL^{-1}) (mean ± range) and density of prymnesiophytes (cells mL^{-1}) (mean ± range) in water samples at Station II in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between chlorophyll-*c3* concentration and prymnesiophyte density at Station II.

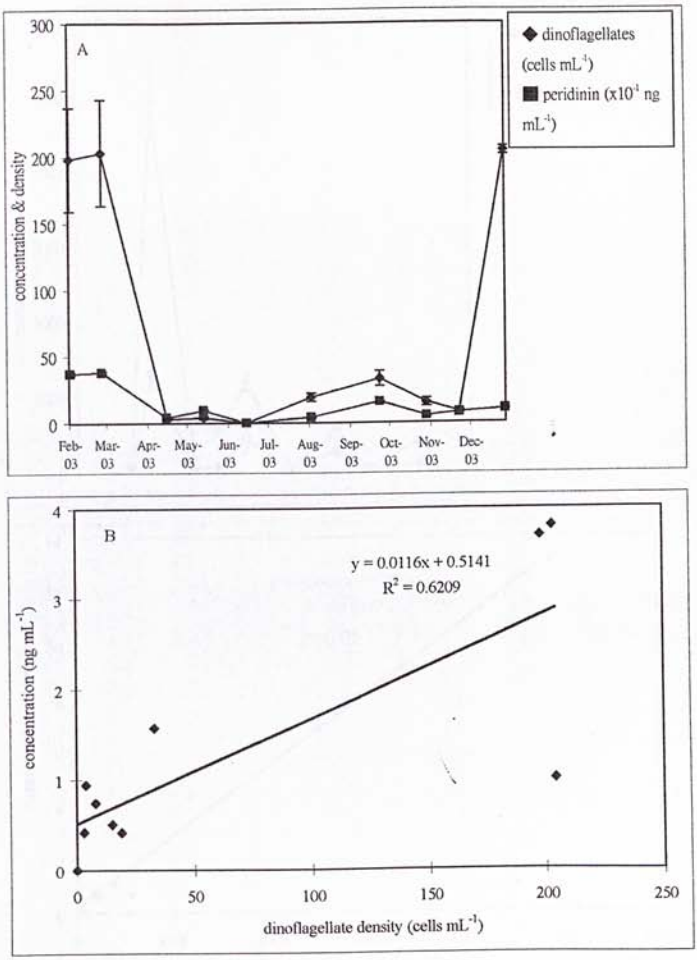


Figure 4.11 A. Temporal changes in peridinin content ($x10^{-1}$ ng mL^{-1}) (mean $\pm$ range) and density of dinoflagellates (cells mL^{-1}) (mean $\pm$ range) in water samples at Station I in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between peridinin concentration and dinoflagellate density at Station I.

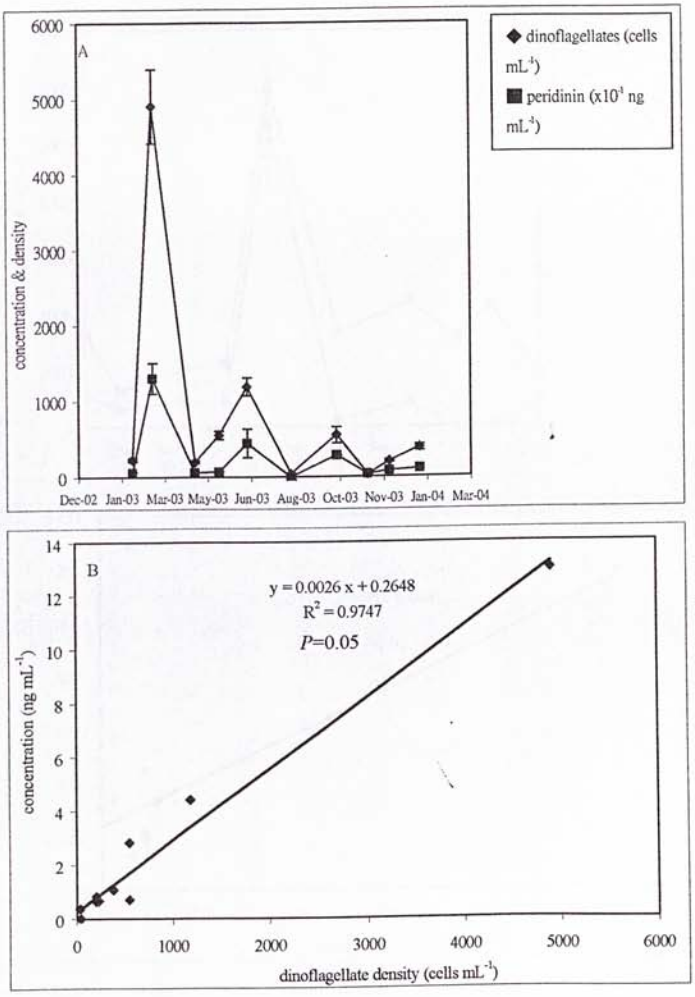


Figure 4.12 A. Temporal changes in peridinin content ($x10^{-2}$ ng mL^{-1}) (mean ± range) and density of dinoflagellates (cells mL^{-1}) (mean ± range) in water samples at Station II in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between peridinin concentration and dinoflagellate density at Station II.

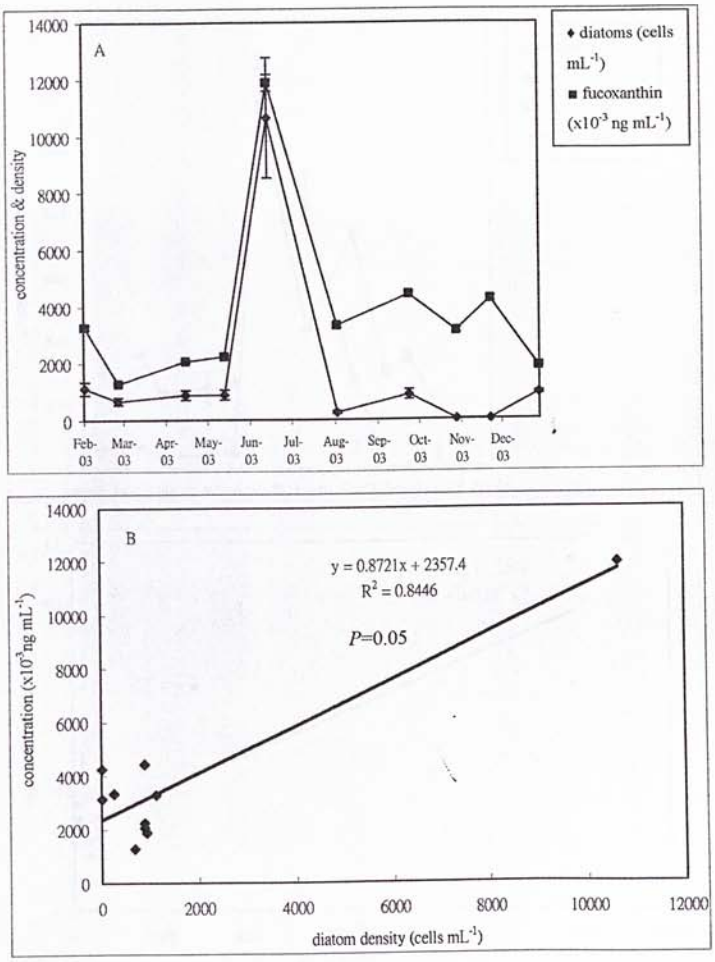


Figure 4.13 A Temporal changes in fucoxanthin content ($x10^{-3}$ ng mL^{-1}) (mean ± range) and density of diatoms (cells mL^{-1}) (mean ± range) in water samples at Station I in Tolo Harbour during the study period in 2003 and 2004. B Relationship between fucoxanthin concentration and diatom density at Station I.

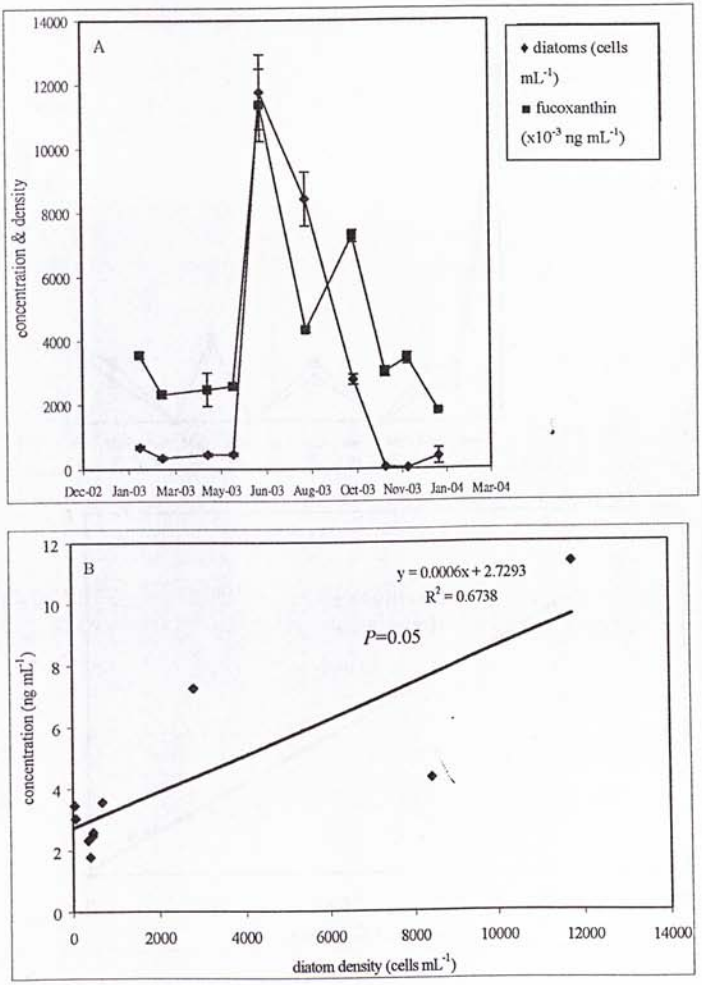


Figure 4.14 A. Temporal changes in fucoxanthin content ($x10^{-3}$ ng mL^{-1}) (mean ± range) and density of diatoms (cells mL^{-1}) (mean ± range) in water samples at Station II in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between fucoxanthin concentration and diatom density at Station II.

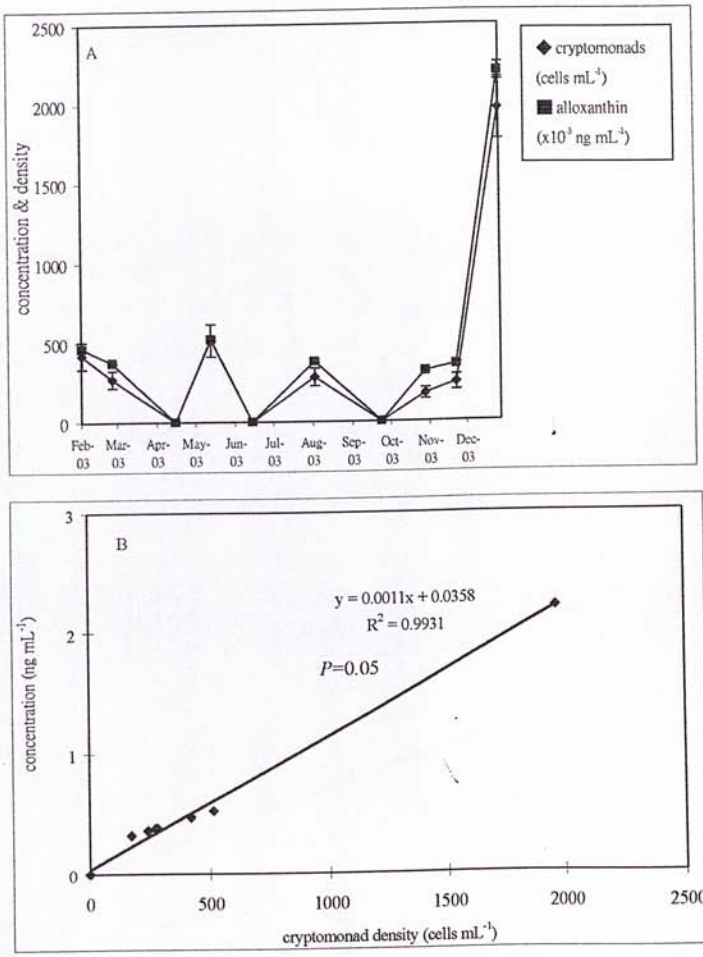


Figure 4.15 A. Temporal changes in alloxanthin content ($x10^{-3}$ ng mL^{-1}) (mean $\pm$ range) and density of cryptomonad (cells mL^{-1}) (mean $\pm$ range) in water samples at Station I in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between alloxanthin concentration and cryptomonad density at Station I.

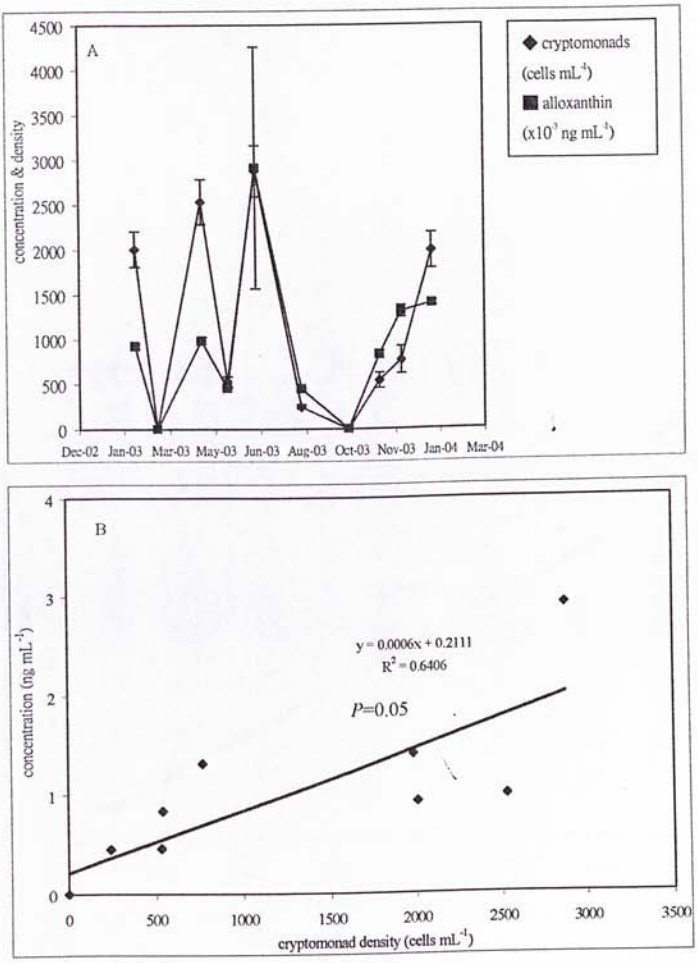


Figure 4.16 A. Temporal changes in alloxanthin content ($x10^{-3}$ ng mL^{-1}) (mean $\pm$ range) and density of cryptomonad (cells mL^{-1}) (mean $\pm$ range) in water samples at Station II in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between alloxanthin concentration and cryptomonad density at Station II.

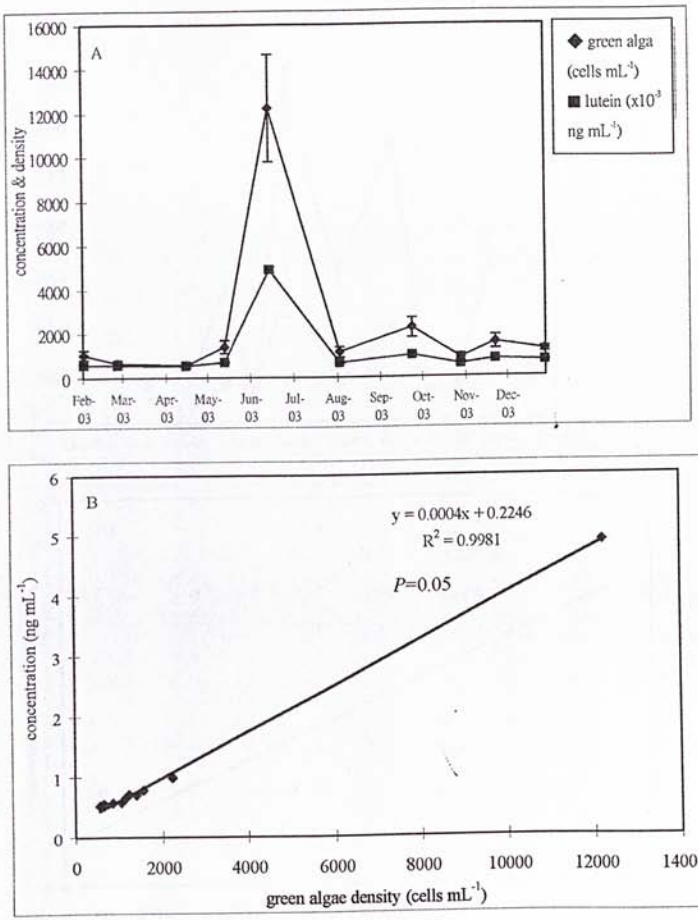


Figure 4.17 A. Temporal changes in lutein content ($x10^{-3}$ ng mL^{-1}) (mean $\pm$ range) and density of green alga (cells mL^{-1}) (mean $\pm$ range) in water samples at Station I in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between lutein concentration and green algae density at Station I.

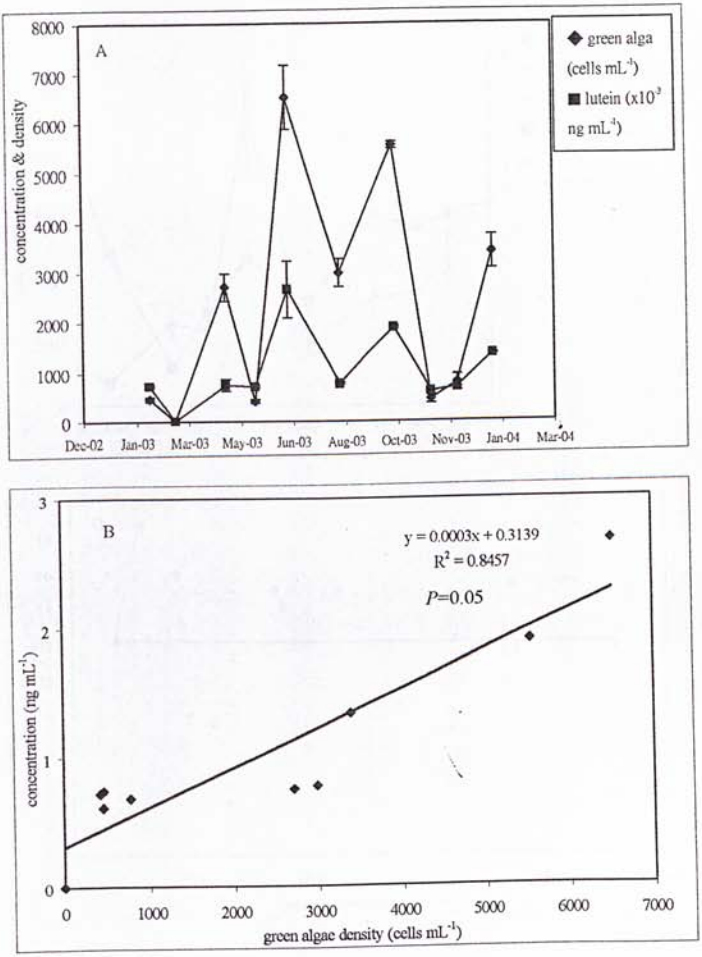


Figure 4.18 A. Temporal changes in lutein content ($x10^{-3}$ ng mL^{-1}) (mean $\pm$ range) and density of green alga (cells mL^{-1}) (mean $\pm$ range) in water samples at Station II in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between lutein concentration and green algae density at Station II.

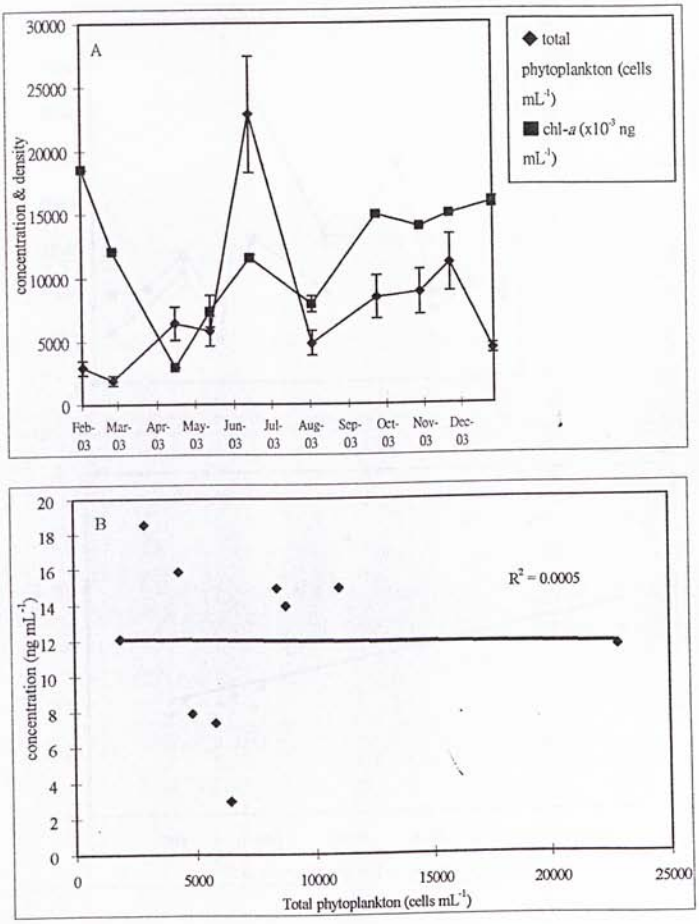


Figure 4.19 A. Temporal changes in chlorophyll-*a* content ($x10^{-3}$ ng mL^{-1}) (mean $\pm$ range) and total phytoplankton cell density (cells mL^{-1}) (mean $\pm$ range) in water samples at Station I in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between chlorophyll-*a* concentration and total phytoplankton cell density at Station I.

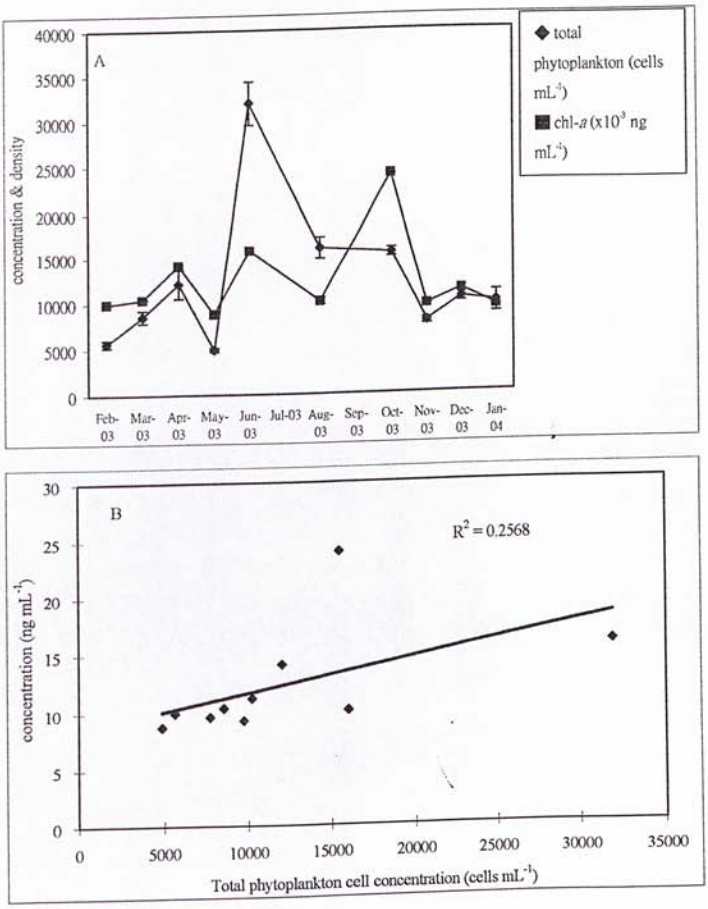


Figure 4.20 A. Temporal changes in chlorophyll-*a* content ($x10^{-3}$ ng mL^{-1}) (mean ± range) and total phytoplankton cell density (cells mL^{-1}) (mean ± range) in water samples at Station II in Tolo Harbour during the study period in 2003 and 2004. B. Relationship between chlorophyll-*a* concentration and total phytoplankton cell density at Station II.

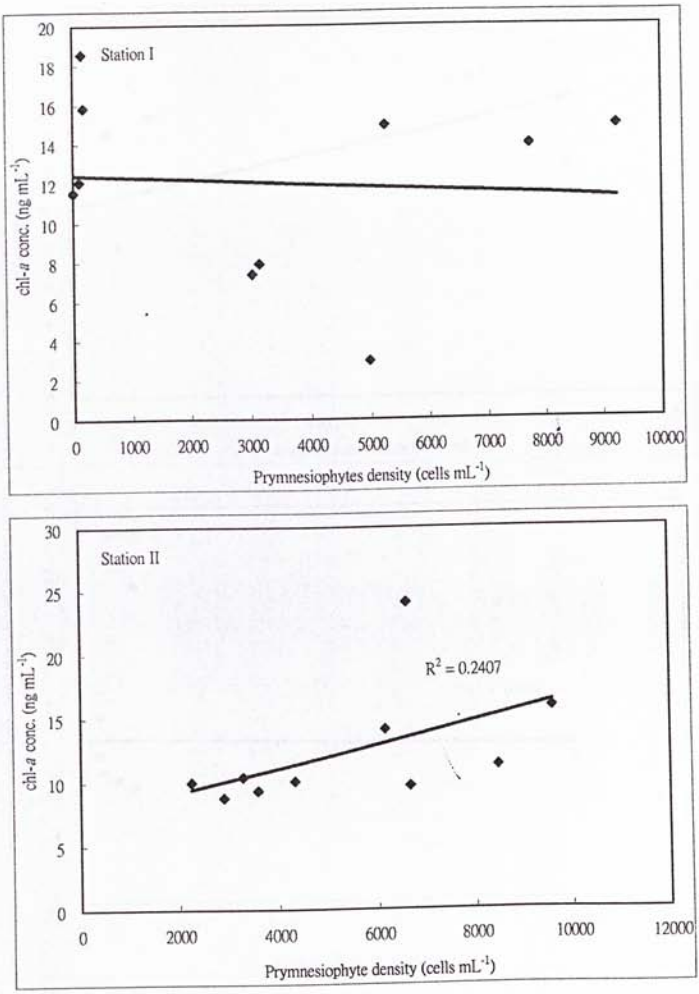


Figure 4.21 Relationship between prymnesiophyte density and chlorophyll-*a* concentration (by HPLC) in water samples collected in Tolo Harbour during the study period in 2003 and 2004.

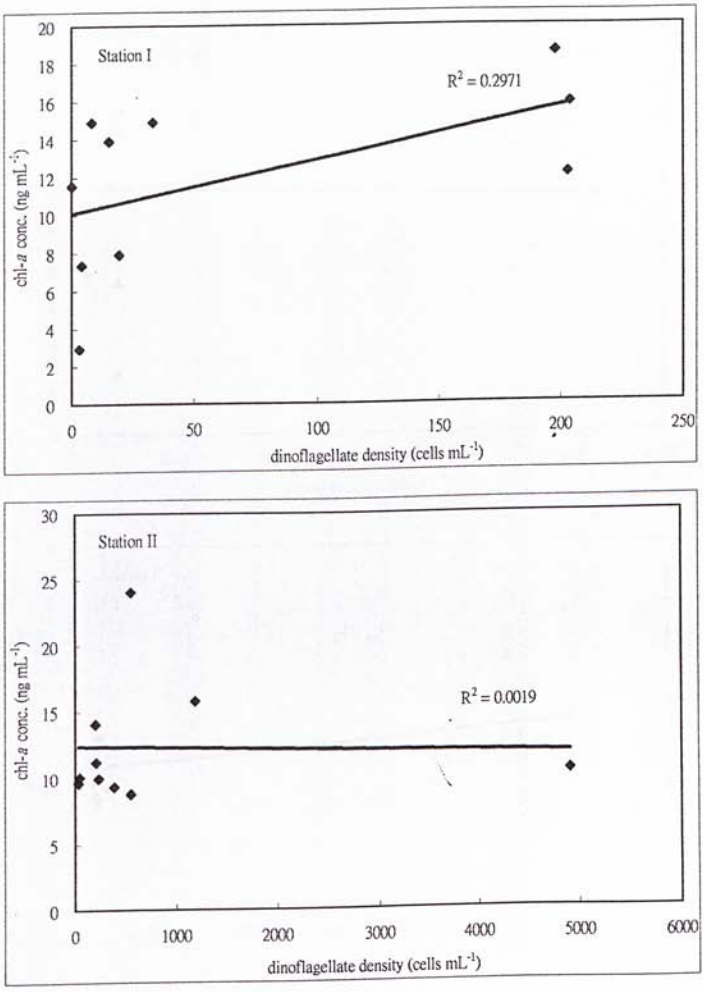


Figure 4.22 Relationship between dinoflagellate density and chlorophyll-*a* concentration (by HPLC) in water samples collected in Tolo Harbour during the study period in 2003 and 2004.

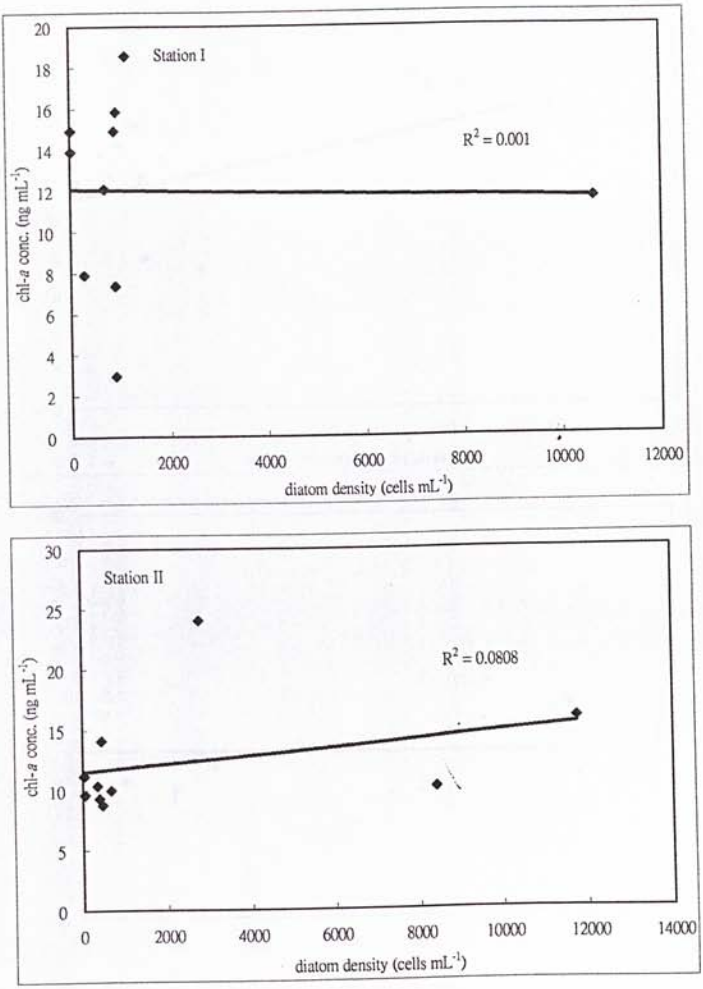


Figure 4.23 Relationship between diatom density and chlorophyll-*a* concentration (by HPLC) in water samples collected in Tolo Harbour during the study period in 2003 and 2004.

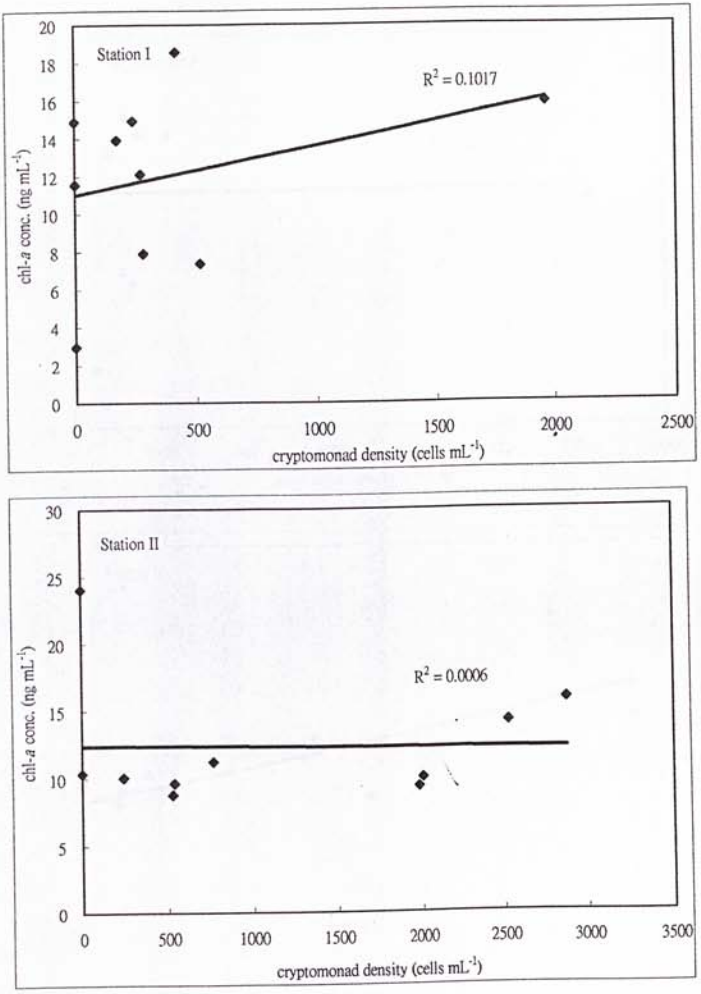


Figure 4.24 Relationship between cryptomonad density and chlorophyll-*a* concentration (by HPLC) in water samples collected in Tolo Harbour during the study period in 2003 and 2004.

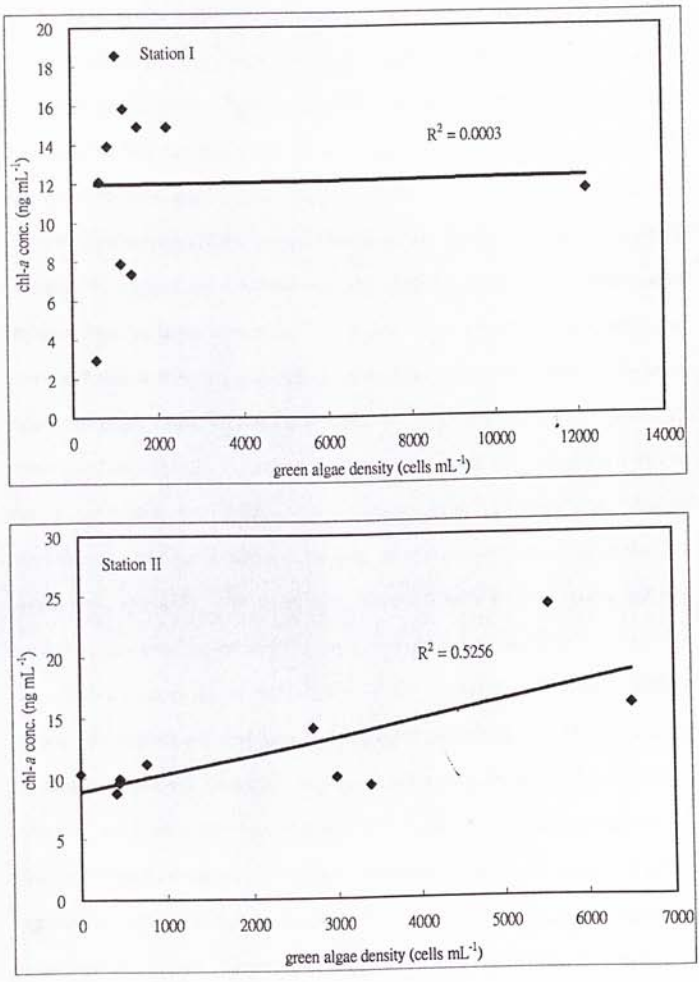


Figure 4.25 Relationship between green algae density and chlorophyll-*a* concentration (by HPLC) in water samples collected in Tolo Harbour during the study period in 2003 and 2004.

4.2.3 Feeding selectivity of zooplankton on different phytoplankton groups

P. avirostris and *Paracalanus* spp. were analyzed by HPLC method for phytoplankton pigments. Figure 4.26 and 4.27 are showing phytoplankton pigments that could be found in guts of *P. avirotris* and *Paracalanus* spp. respectively. The above two chromatographs show the pigments that could be extracted in guts of *P. avirostris* in Feburary 2003 at Station I and in guts of *Paracalanus* spp. in June 2003 at Station II, respectively. In these two zooplankton samples, most of the possible phytoplankton pigments were found in the guts. That meant these zooplankton ate most of the phytoplankton populations rather than just a few of them. Therefore, these two graphs were chosen for presentation. In the guts of *P. avirostris*, the presence of chlorophyll-*c3*, peridinin, fucoxanthin, alloxanthin and lutein indicated that *P. avirostris* was feeding on prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green algae. In guts of *Paracalanus* spp., chlorophyll-*c3*, fucoxanthin, alloxanthin and lutein were found. Therefore, *Paracalanus* spp. was feeding on prymnesiophytes, diatoms, cryptomonads and green algae.

The relative proportions of the various marker pigments for the phytoplankton groups (chlorophyll-*c3*, peridinin, fucoxanthin, neoxanthin, 19-hex-fucoxanthin, alloxanthin, lutein and chlorophyll *b*) were determined for phytoplankton samples, *P. avirostris* and *Paracalanus* spp. (Figure 4.28 – 4.32). Results for zooplankton guts with or without correction of pigment degradation were also shown. Pigment degradations in zooplankton guts were corrected according to pigment degradation percentages of different pigment markers after ingestion estimated in zooplankton feeding experiments presented in Section 4.3. The proportions of the various marker pigments in phytoplankton and zooplankton were compared using *t*-test (Table 4.2). Results of *t*-tests were not the same for data corrected with pigment degradation and not corrected with pigment degradation. Significant differences between proportions

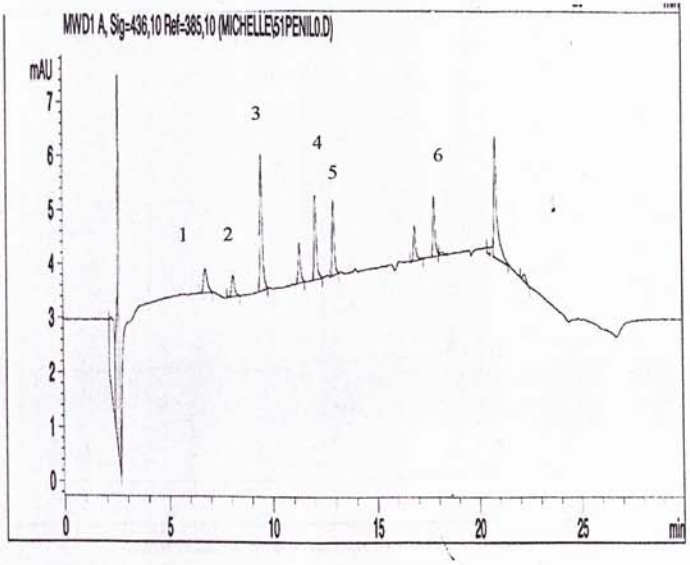


Figure 4.26 Chromatograph for *P. avirostris* collected in February 2003 at Station I (1: chlorophyll-*c3*; 2: peridinin; 3: fucoxanthin; 4: alloxanthin; 5: lutein & 6: chlorophyll-*a*).

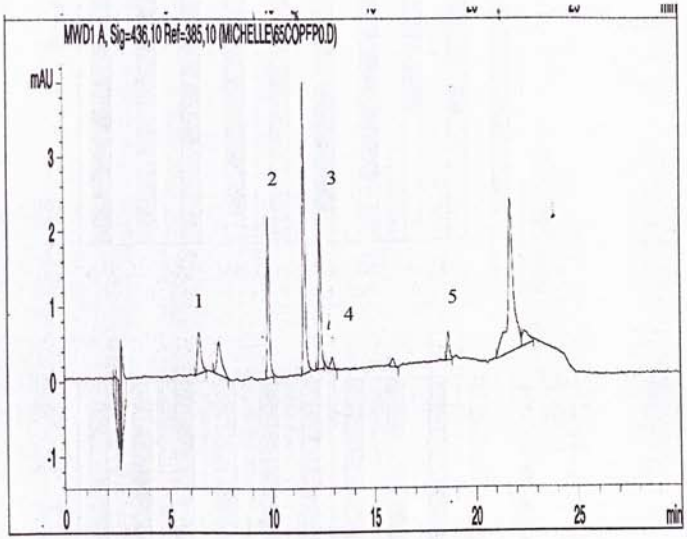


Figure 4.27 Chromatograph for *Paracalanus* spp. collected in June 2003 at Station II (1: chlorophyll-*c3*; 2: fucoxanthin; 3: alloxanthin; 4: lutein & 5: chlorophyll-*a*).

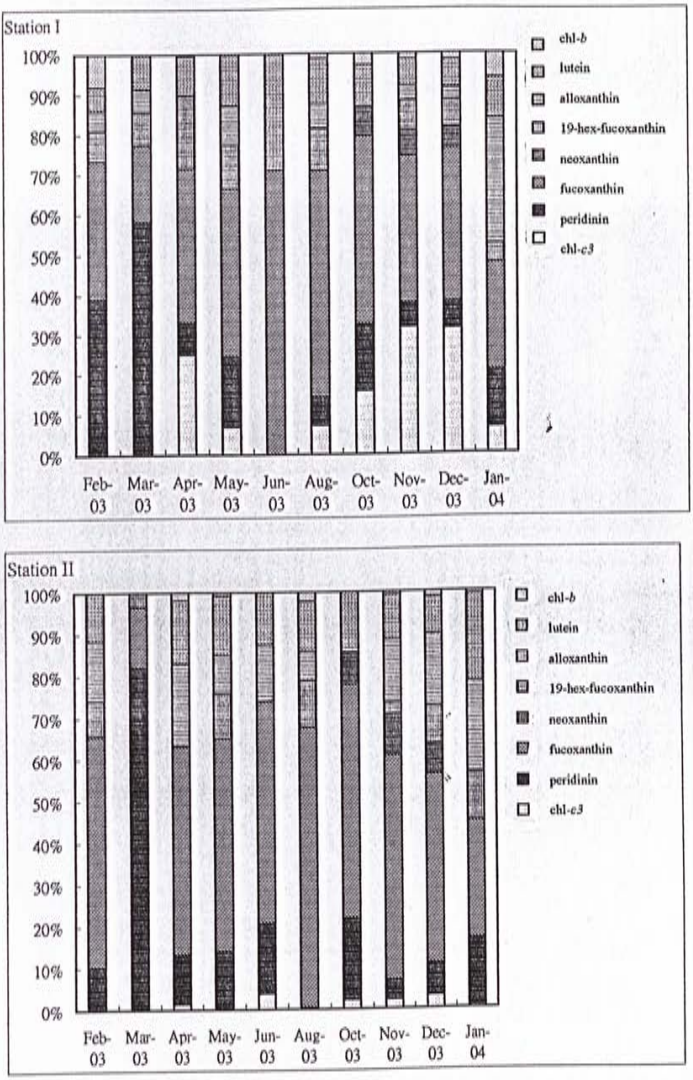


Figure 4.28 Phytoplankton pigment markers (expressed as percentage of the sum of all pigments) in water samples.

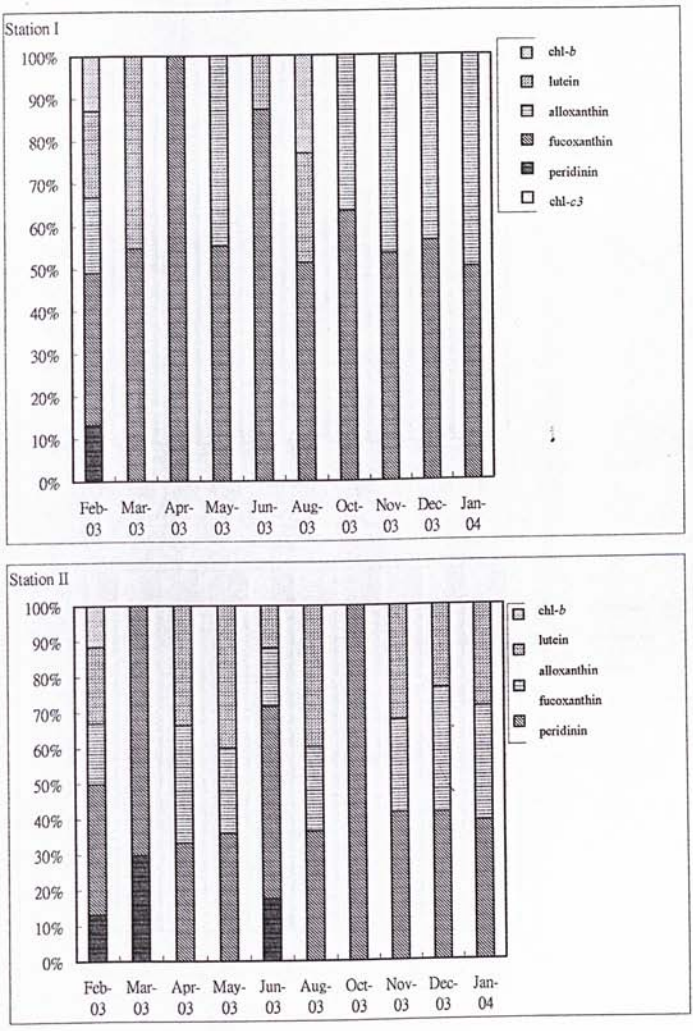


Figure 4.29 Phytoplankton pigment markers (expressed as percentage of the sum of all pigments) in *P. avirostris* (without correction for pigment degradation).

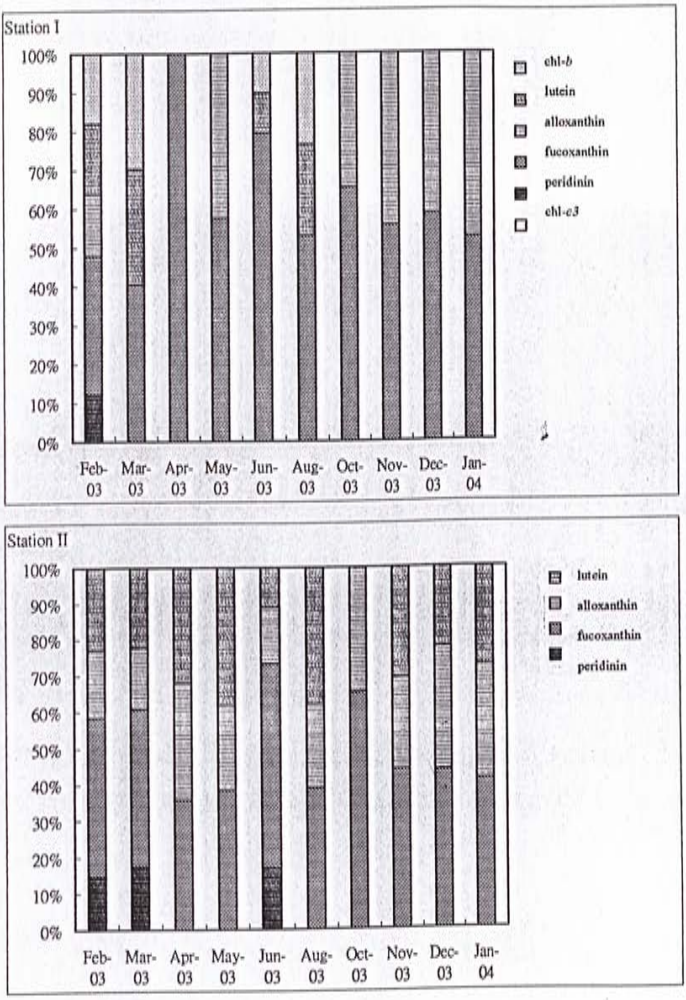


Figure 4.30 Phytoplankton pigment markers (expressed as percentage of the sum of all pigments) in *P. avirostris* (with correction of pigment degradation). (Please refer to Table 4.7 for the degree of pigment degradation correction.)

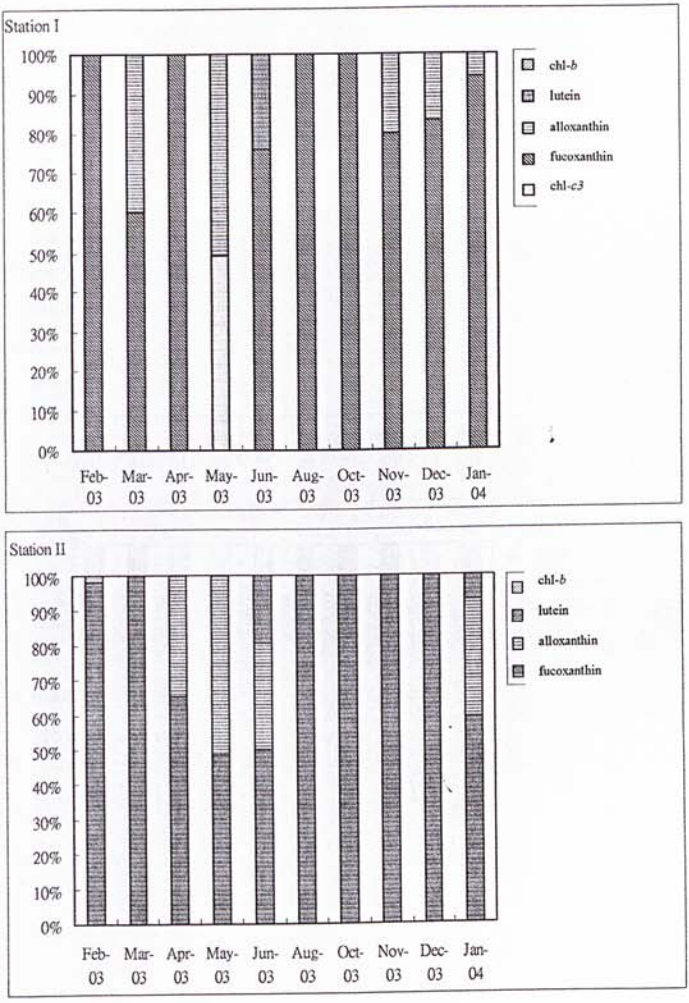


Figure 4.31 Phytoplankton pigment markers (expressed as percentage of the sum of all pigments) in *Paracalanus* spp. (without correction of pigment degradation). (Please refer to Table 4.7 for the degree of pigment degradation correction.)

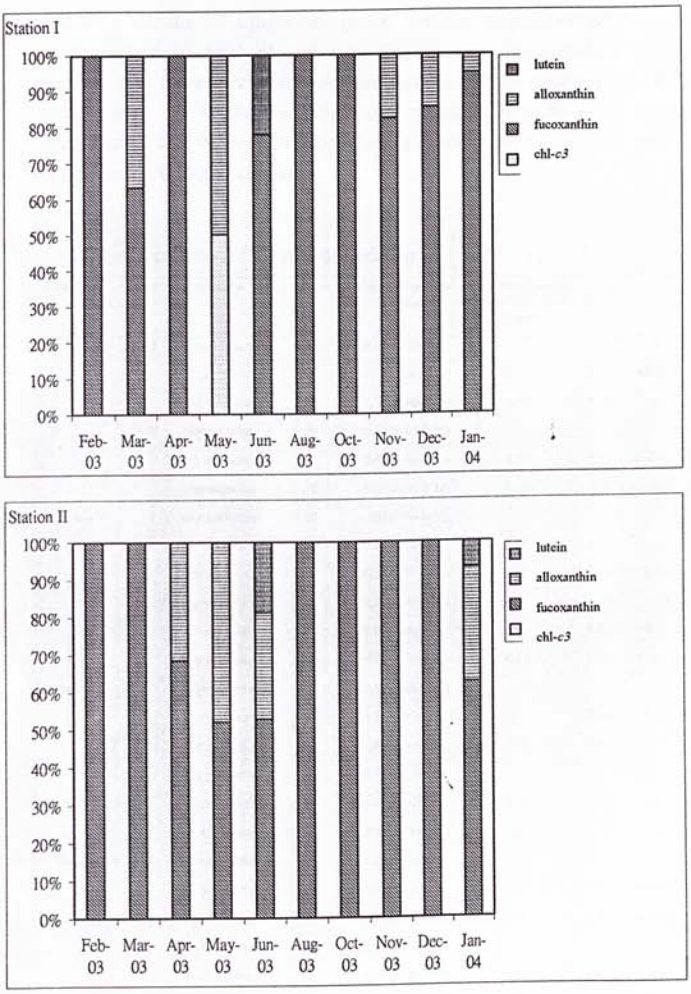


Figure 4.32 Phytoplankton pigment markers (expressed as percentage of the sum of all pigments) in *Paracalanus* spp. (with correction of pigment degradation). (Please refer to Table 4.7 for the degree of pigment degradation correction.)

Table 4.2 Results of comparison (t-test) between proportions of the main pigment markers to total phytoplankton pigments in phytoplankton and in zooplankton guts (data were arcsine transformed). Data were compared for all the sampling dates. $t < 0$: pigment proportions are higher in zooplankton than in phytoplankton. $t > 0$: pigment proportions are lower in zooplankton than in phytoplankton. (Not significant-ns)

A Without correction of pigment degradation

Pigments	Station	Samples	No. of samples	Mean proportions (range)	Mean proportions in zooplankton to that in phytoplankton	*t*	*P*
chlorophyll-*c*3	I	phytoplankton	10	0.17 (0.00-0.34)			
		P. avirostris	10	0.02 (0.00-0.18)	0.12	2.49	<0.05
		Paracalamus	10	0.04 (0.00-0.45)	0.24	1.70	ns
	II	phytoplankton	10	0.20 (0.09-0.33)			
		P. avirostris	10	0.00 (0.00-0.00)	0.00	7.51	<0.0001
		Paracalamus	10	0.02 (0.00-0.22)	0.10	5.28	<0.0005
peridinin	I	phytoplankton	10	0.22 (0.00-0.50)			
		P. avirostris	10	0.02 (0.00-0.20)	0.09	4.35	<0.005
		Paracalamus	10	0.00 (0.00-0.00)	0.00	4.39	<0.01
	II	phytoplankton	10	0.22 (0.00-0.63)			
		P. avirostris	10	0.07 (0.00-0.25)	0.32	3.05	<0.01
		Paracalamus	10	0.03 (0.00-0.26)	0.14	3.02	<0.01
fucoxanthin	I	phytoplankton	10	0.40 (0.26-0.57)			
		P. avirostris	10	0.53 (0.35-0.90)	1.33	-2.43	<0.05
		Paracalamus	10	0.68 (0.00-0.90)	1.7	-2.65	<0.05
	II	phytoplankton	10	0.39 (0.22-0.52)			
		P. avirostris	10	0.40 (0.35-0.52)	1.03	-0.14	ns
		Paracalamus	10	0.72 (0.35-0.90)	1.85	-3.98	<0.005
19-hex-fucoxanthin	I	phytoplankton	10	0.15 (0.00-0.25)			
		P. avirostris	10	0.00 (0.00-0.00)	0.00	7.03	<0.0005
		Paracalamus	10	0.00 (0.00-0.00)	0.00	7.03	<0.0001
	II	phytoplankton	10	0.11 (0.00-0.19)			
		P. avirostris	10	0.00 (0.00-0.00)	0.00	3.61	<0.005
		Paracalamus	10	0.00 (0.00-0.00)	0.00	3.61	<0.005
alloxanthin	I	phytoplankton	10	0.12 (0.00-0.34)			
		P. avirostris	10	0.23 (0.00-0.45)	1.92	-1.77	ns
		Paracalamus	10	0.15 (0.00-0.45)	1.25	-1.19	ns
	II	phytoplankton	10	0.16 (0.00-0.23)			
		P. avirostris	10	0.31 (0.25-0.37)	1.94	-4.40	<0.005
		Paracalamus	10	0.14 (0.00-0.46)	0.88	0.51	ns

lutein	I	phytoplankton	10	0.19 (0.15-0.34)			
		P. avirostris	10	0.12 (0.00-0.42)	0.63	1.04	ns
		Paracalanus	10	0.03 (0.00-0.29)	0.16	9.12	<0.0001
	II	phytoplankton	10	0.18 (0.00-0.21)			
		P. avirostris	10	0.29 (0.00-0.39)	1.61	-2.29	<0.05
		Paracalanus	10	0.04 (0.00-0.21)	0.22	4.34	<0.001
chlorophyll-*b*	I	phytoplankton	10	0.07 (0.00-0.16)			
		P. avirostris	10	0.04 (0.00-0.29)	0.57	0.48	ns
		Paracalanus	10	0.00 (0.00-0.00)	0.00	3.10	<0.01
	II	phytoplankton	10	0.06 (0.00-0.18)			
		P. avirostris	10	0.02 (0.00-0.20)	0.33	0.96	ns
		Paracalanus	10	0.01 (0.00-0.06)	0.17	2.03	<0.05

B With correction of pigment degradation

Pigments	Station	Samples	No of samples	Mean proportions (range)	Mean proportions in zooplankton to that in phytoplankton	*t*	*P*
chlorophyll-*c3*	I	phytoplankton	10	0.17 (0.00-0.34)			
		P. avirostirs	10	0.02 (0.00-0.20)	0.12	2.68	<0.05
		Paracalanus	10	0.05 (0.00-0.45)	0.29	1.92	<0.05
	II	phytoplankton	10	0.20 (0.09-0.32)			
		P. avirostirs	10	0.00 (0.00-0.00)	0.00	8.26	<0.00001
		Paracalanus	10	0.00 (0.00-0.00)	0.00	4.34	<0.001
peridinin	I	phytoplankton	10	0.22 (0.00-0.50)			
		P. avirostirs	10	0.02 (0.00-0.21)	0.09	4.34	<0.0005
		Paracalanus	10	0.00 (0.00-0.00)	0.00	5.02	<0.0005
	II	phytoplankton	10	0.22 (0.00-0.63)			
		P. avirostirs	10	0.07 (0.00-0.25)	0.32	3.49	<0.005
		Paracalanus	10	0.00 (0.00-0.00)	0.00	4.34	<0.001
fucoxanthin	I	phytoplankton	10	0.40 (0.26-0.57)			
		P. avirostirs	10	0.55 (0.38-0.90)	1.38	-3.58	<0.005
		Paracalanus	10	0.68 (0.00-0.90)	1.70	-3.18	<0.01
	II	phytoplankton	10	0.39 (0.22-0.52)			
		P. avirostirs	10	0.42 (0.41-0.53)	1.08	-0.96	ns
		Paracalanus	10	0.74 (0.42-0.90)	1.90	-4.34	<0.001
19-hex-fucoxanthin	I	phytoplankton	10	0.15 (0.00-0.25)			
		P. avirostirs	10	0.00 (0.00-0.00)	0.00	7.58	<0.0001
		Paracalanus	10	0.00 (0.00-0.00)	0.00	7.58	<0.0001
	II	phytoplankton	10	0.11 (0.00-0.19)			
		P. avirostirs	10	0.00 (0.00-0.00)	0.00	4.33	<0.005
		Paracalanus	10	0.00 (0.00-0.00)	0.00	4.33	<0.005

alloxanthin	I	phytoplankton	10	0.12 (0.00-0.34)			
		P. avirostirs	10	0.24 (0.00-0.50)	2.00	-1.96	<0.05
		Paracalanus	10	0.14 (0.00-0.45)	1.17	-0.55	ns
	II	phytoplankton	10	0.16 (0.00-0.27)			
		P. avirostirs	10	0.30 (0.23-0.36)	1.88	-4.39	<0.001
		Paracalanus	10	0.14 (0.00-0.44)	0.88	0.42	ns
lutein	I	phytoplankton	10	0.19 (0.15-0.33)			
		P. avirostirs	10	0.12 (0.00-0.41)	0.63	1.33	ns
		Paracalanus	10	0.03 (0.00-0.28)	0.16	11.0	<0.0001
	II	phytoplankton	10	0.18 (0.00-0.21)			
		P. avirostirs	10	0.28 (0.00-0.38)	1.56	-2.34	<0.05
		Paracalanus	10	0.03 (0.00-0.23)	0.17	5.17	<0.0005
chlorophyll-*b*	I	phytoplankton	10	0.07 (0.00-0.16)			
		P. avirostirs	10	0.00 (0.00-0.00)	0.00	3.52	<0.005
		Paracalanus	10	0.00 (0.00-0.00)	0.00	3.52	<0.005
	II	phytoplankton	10	0.08 (0.00-0.25)			
		P. avirostirs	10	0.00 (0.00-0.00)	0.00	2.76	<0.05
		Paracalanus	10	0.00 (0.00-0.00)	0.00	2.76	<0.05

of alloxanthin at Station I and chlorophyll *b* at both stations in phytoplankton and *P. avirostris* were resulted after correction of pigment degradation. Significant differences also resulted for chlorophyll-*c3* in *Paracalanus* spp. at Station I. In later sections of this thesis, only data corrected with pigment degradation will be used for drawing results and conclusion on the feeding selectivity of zooplankton.

From Table 4.2, *P. avirostris* selected for diatoms, cryptomonads and green alga significantly. Positive selections of *P. avirostris* were significant on cryptomonads at both stations, but only significant on diatoms and green alga at one station. Dinoflagellates and prymnesiophytes were significantly selected against by *P. avirostris*. Negative selections on dinoflagellates and prymnesiophytes for *P. avirostris* were significant at both sampling stations. For *Paracalanus* spp., they selected for diatoms but against prymnesiophytes, dinoflagellates and green alga significantly. Selection patterns were similar at both stations. For cryptomonads, *Paracalanus* spp. didn't show any positive or negative selections.

Although positive selectivity of *P. avirostris* on diatoms was not significant at Station II, diatoms were eaten by *P. avirostris* throughout the year regardless of the abundance of diatoms. However, selectivity of *P. avirostris* on cryptomonads and green alga changed with the changes in plankton composition temporally. In March and August at Station I, no alloxanthin was detected in guts of *P. avirostris* indicating no ingestion of cryptomonads even with the presence of cryptomonads in the sea. Lutein were not detected in guts of *P. avirostris* in October at Station II even though there was a general significant positive selection by *P. avirostris* on green alga throughout the year at this station. At Station I, there were even six months in which no lutein was detected in guts of *P. avirostris* (Figure 4.29 & 4.30).

Negative selections on dinoflagellaltes and prymnesiophytes were exhibited by *P. avirostris*. No chlorophyll-*c3* was detected in guts of *P. avirostris* at both

sampling stations throughout the year. However, *P. avirostris* exhibited a selection for dinoflagellates when most the dinoflagellate community was dominated by *Protocentrum* spp. Selectivity by *P. avirostris* on dinoflagellates was weak when the dinoflagellate community was dominated by *Scrippsiella* spp. or other dinoflagellates.

Paracalanus spp. showed strong selection for diatoms at both sampling station throughout the year except May at Station I in which no diatoms were detected in guts of *Paracalanus* spp. Generally speaking, *Paracalanus* spp. selected against pymnesiophytes, dinoflagellates and green alga significantly throughout the year. This was absolutely true for dinoflagellates as no peridinin was detected in guts of *Paracalanus* spp. at both sampling stations throughout the year regardless of the abundance and dominant species of dinoflagellates. However, chlorophyll-*c3* and lutein were detected in guts of *Paracalanus* spp. in some months. Chlorophyll-*c3* was detected in guts of *Paracalanus* spp. in May at Station I. Proportions of chlorophyll-*c3* in guts of *Paracalanus* spp. were higher than that in seawater. Lutein was detected in guts of *Paracalanus* spp. in June at both sampling stations and in January at Station II. However, the proportions of lutein in guts of *Paracalanus* spp. for these three samples were about the same or less than that in seawater. For cryptomonads, no significant selections for or against this phytoplankton group were demonstrated by *Paracalanus* spp. However, there was selection for cryptomonads by *Paracalanus* spp. in some months at some stations and there was also selection against cryptomonads by *Paracalanus* spp. in some months at some stations. A higher proportion of alloxanthin in guts of *Paracalanus* spp. relative to that in seawater were found in March, May, November and December at Station I and in April May, June and January at Station II. A lower proportion or a zero proportion of alloxanthin in guts of *Paracalanus* spp. relative to that in seawater were found in

February and August at Station I and in February, August, November and December at Station II.

4.2.4 Feeding selectivity of zooplankton on dinoflagellates

In general, both *P. avirostris* and *Paracalanus* spp. selected against dinoflagellates. That means the relative proportions of peridinin were significantly lower in their guts than in seawater. There was even no detectable peridinin (pigment specific for dinoflagellates) in *Paracalanus* spp.

Although *P. avirostris* also selected against dinoflagellates and no detectable peridinin was found in guts of *P. avirostris* at most times, peridinin was detected in some months (Figures 4.19 and 4.30). In February, peridinin was detected in guts of *P. avirostris* at both sampling stations. In this month, the dominant dinoflagellate species was *Prorocentrum* spp. (Figure 4.5). The cell concentrations of *Prorocentrum* spp. were 198 and 210 cells mL^{-1} at Station I and Station II, respectively. These results were consistent throughout the study period as peridinin was also detected in guts of *P. avirostris* when concentrations of *Prorocentrum* spp. were high in March and June 2003. In March, concentration of dinoflagellates was high in Tolo Harbour. However, no detectable peridinin was found in guts of *P. avirostris* at Station I. This was because concentration of *Prorocentrum* spp. was not high enough (<200 cells mL^{-1}). There was no evidence, based on gut pigment levels, that the bloom of *Scrippsiella* sp. at Station I was consumed by *P. avirostris*.

4.2.5 Feeding selectivity of zooplankton on diatoms

Both *P. avirostris* and *Paracalanus* spp. selected for diatoms as food. Cell concentrations of different diatom populations throughout the year in Tolo Harbour were shown in Figure 4.6. The diatom populations included Coscinodicales, Biddulphiales, Rhizosoleniales, Naviculales, Diatomales, Phaeodactylales and Surirellales. Common species of diatoms were *Asterionellopsis glacialis*, *Cerataulina pelagica*, *Chaetoceros* spp., *Coscinodiscus* spp. *Guinardia* spp.,

Leptocylindrus danicus, *Pseudonitzschia* spp., *Skeletonema costatum*, *Thalassionema* spp., and *Thalassiosira* spp.

4.3 Feeding selectivity on phytoplankton by other cladoceran - *Pseudevadne tergestina*

Large numbers of the podonid cladoceran *P. tergestina* were found in October 2003 at Station I in Tolo Harbour. On average, 0.15 ng of fucoxanthin was detected in the gut of each individual *P. tergestina.* The presence of fucoxanthin in the guts suggests that diatoms were ingested by *P. tergestina.*

4.4 Pigment degradation in zooplankton guts after ingestion of phytoplankton

According to Section 3.2.5, pigment degradations in zooplankton guts after phytoplankton ingestion were estimated. The results were shown in Tables 4.3 & 4.4. The numbers of replicates for each pigment varied because not all pigments were present in the natural seawater collected for each experimental day. The mean pigment degradation rates in *P. avirostris* ranged from 9.78% for lutein to 24.3% for chlorophyll-*a*. The mean pigment degradation rates in *Paracalanus* spp. were from 11.75% for peridinin to 29.37% for chlorophyll-*a*. Table 4.5 presents a summary of the mean pigment degradations rates of both species.

Table 4.3 Pigment degradations in guts of *P. avirostris* after phytoplankton ingestion. K_0: phytoplankton cell concentration at time 0 in the bottle (cells mL^{-1}); K_τ: phytoplankton cell concentration after grazing by zooplankton for 18 hr (cell mL^{-1}); C_0: pigment concentration in water at time 0 (ng mL^{-1}); C_{18}: pigment concentration in water at time 18hr (ng mL^{-1}); C_τ: pigment concentration in water at time 18hr after subtraction of fecal pigment (ng mL^{-1}); Δ: pigment ingested (ng); S_f: remaining phytoplankton pigment in feace (ng); D*: pigment degradation efficiency, $[(\Delta - S_f)/\Delta]\%$. (For culture media, seawater means natural seawater collected from the field filtered through filter with 125μm pore size. Chrysophytes means natural seawater filtered through 47 mm diameter Whatman GF/C filters with laboratory cultured chrysophytes added.)

Pigments	Dates	Culture media	K_0	K_τ	C_0	C_{18}	$C_\tau = C_0(K_\tau/K_0)$	$\Delta = C_0 - C_\tau$	$S_f = C_{18} - C_\tau$	D*
chlorophyll-*c3*	3/Jul/2003	seawater	197	0	0.46	0.33	0.00	0.46	0.33	28.3
			198	0	0.46	0.33	0.00	0.46	0.33	28.3
	30/Jul/2003		606	105	1.41	1.39	0.24	1.17	1.15	1.72
			696	375	1.62	1.54	0.87	0.75	0.67	10.7
	2/Oct/2003		1425	980	1.45	1.43	1.00	0.45	0.43	4.33
			1475	890	1.48	1.45	0.89	0.59	0.56	5.11
	6/Nov/2003		7723	7324	2.73	2.70	2.59	0.14	0.11	21.3
			7749	7342	2.73	2.70	2.59	0.14	0.11	20.9
peridinin	30/Jul/2003	seawater	43	0	2.29	2.27	0.00	2.29	2.27	0.87
			40	0	2.25	1.96	0.00	2.25	1.96	12.9
	2/Oct/2003		422	382	2.90	2.85	2.63	0.27	0.22	18.2
			492	458	3.00	2.95	2.79	0.21	0.16	24.3
	6/Nov/2003		16	16	0.51	0.51	0.51	0.00	0.00	-
			17	17	0.52	0.52	0.52	0.00	0.00	-

Table 4.3 (continued)

Pigments	Dates	Culture media	K_0	K_τ	C_0	C_{18}	$C_\tau = C_0(K_\tau/K_0)$	$\Delta = C_0 - C_\tau$	$S_f = C_{18} - C_\tau$	D*
fucoxanthin	3/Jul/2003	seawater	405	0	4.34	4.50	0.00	4.34	4.50	7.63
			401	0	4.34	4.42	0.00	4.34	4.42	8.87
	30/Jul/2003		685	105	0.91	0.83	0.14	0.77	0.69	10.4
			775	375	0.91	0.91	0.44	0.47	0.47	0.00
	2/Oct/2003		1535	1300	5.32	5.21	4.41	0.81	0.70	13.5
			2254	1352	4.33	4.34	2.94	1.96	1.92	2.04
	6/Nov/2003		7749	7301	3.14	3.09	2.96	0.18	0.13	27.5
			7757	7336	3.14	3.08	2.97	0.17	0.11	35.2
	3/Jul/2003	chrysophytes	55097	44574	16.7	15.4	13.5	3.19	1.89	40.8
			55265	44520	16.9	15.4	13.6	3.29	1.79	45.7
	30/Jul/2003		1485	0	9.58	7.44	0.00	9.58	7.44	22.3
			1485	0	9.58	6.51	0.00	9.58	6.51	32.1
	2/Oct/2003		1865	1792	9.88	9.75	9.49	0.39	0.26	33.6
			1798	1703	9.39	9.25	8.89	0.50	0.36	28.2
	6/Nov/2003		600	120	5.23	5.01	1.05	4.33	3.96	5.26
			590	110	5.12	5.00	0.95	4.33	4.05	2.88
19-hex-fucoxanthin	30/Jul/2003	seawater	606	105	0.66	0.65	0.11	0.55	0.54	1.83
			696	375	0.76	0.67	0.41	0.35	0.26	25.7
	2/Oct/2003		1425	980	0.80	0.76	0.55	0.25	0.21	16.0
			1475	890	0.76	0.73	0.46	0.30	0.27	9.95
	6/Nov/2003		7723	7324	0.61	0.60	0.58	0.03	0.02	31.7
			7749	7342	0.62	0.61	0.59	0.03	0.02	30.7

Table 4.3 (continued)

Pigments	Dates	Culture media	K_0	K_r	C_0	C_{18}	$C_r = C_0(K_r/K_0)$	$\Delta = C_0 - C_r$	$S_f = C_{18} - C_r$	D*
alloxanthin	30/Jul/2003	seawater	331	58	0.37	0.36	0.06	0.31	0.30	3.28
			412	139	0.46	0.42	0.16	0.30	0.26	13.1
	6/Nov/2003		173	36	0.32	0.28	0.07	0.25	0.21	15.8
			168	34	0.31	0.27	0.06	0.25	0.21	16.2
lutein	3/Jul/2003	seawater	730	162	0.84	0.84	0.19	0.65	0.65	0.00
			752	184	0.83	0.83	0.20	0.63	0.63	0.00
	30/Jul/2003		689	121	0.76	0.76	0.13	0.63	0.63	0.00
			734	166	0.81	0.76	0.18	0.63	0.58	7.98
	2/Oct/2003		1486	1025	1.18	1.15	0.81	0.37	0.34	8.20
			1412	986	1.12	1.09	0.78	0.34	0.31	8.88
	6/Nov/2003		834	450	0.57	0.49	0.31	0.26	0.18	30.5
			830	430	0.56	0.49	0.29	0.27	0.20	25.9

Table 4.3 (continued)

Pigments	Dates	Culture media	K_0	K_r	C_0	C_{18}	$C_r = C_0(K_r/K_0)$	$\Delta = C_0 - C_r$	$S_f = C_{18} - C_r$	D*
chlorophyll-*a*	3/Jul/2003	seawater	1135	162	12.9	12.0	1.84	11.1	10.2	8.14
			1153	184	13.0	12.1	2.07	10.9	10.0	8.24
	30/Jul/2003		1748	284	5.05	5.05	0.82	4.33	4.33	0.00
			1961	680	4.37	3.95	1.55	2.92	2.40	17.8
	2/Oct/2003		3443	2707	18.1	17.9	14.3	3.87	3.67	5.17
			4158	2796	17.2	17.0	11.6	5.63	5.43	3.55
	6/Nov/2003		8772	7803	13.9	13.6	12.4	1.54	1.19	22.8
			8772	7817	13.9	13.6	12.4	1.51	1.19	21.2
	3/Jul/2003	chrysophytes	55097	44574	57.1	49.6	46.2	10.9	3.41	68.8
			55265	44520	57.1	49.8	46.0	11.1	3.80	65.8
	30/Jul/2003		1485	0	27.9	20.9	0.00	27.9	20.9	25.0
			1485	0	27.9	15.7	0.00	27.9	15.7	43.7
	2/Oct/2003		1865	1792	30.1	29.6	28.9	1.18	0.68	42.4
			1798	1703	28.6	27.8	27.1	1.51	0.71	52.9
	6/Nov/2003		600	120	20.1	19.8	4.02	16.1	15.8	1.99
			590	110	20.1	19.9	3.75	16.4	16.1	1.28

Table 4.4 Pigment degradations in guts of *Paracalanus* spp. after phytoplankton ingestion. K_0: phytoplankton cell concentration at time 0 in the bottle (cells mL^{-1}); K_τ: phytoplankton cell concentration after grazing by zooplankton for 18 hr (cell mL^{-1}); C_0: pigment concentration in water at time 0 (ng mL^{-1}); C_{18}: pigment concentration in water at time 18hr (ng mL^{-1}); C_τ: pigment concentration in water at time 18hr after subtraction of fecal pigment (ng mL^{-1}); Δ: pigment ingested (ng); S_f: remaining phytoplankton pigment in feace (ng); D*: pigment degradation efficiency, [(Δ-Sf)/Δ]%. (For culture media, seawater means natural seawater collected from the field filtered through filter with 125μm pore size. Chrysophytes means natural seawater filtered through 47 mm diameter Whatman GF/C filters with laboratory cultured chrysophytes added.)

Pigments	Dates	culture media	K_0	K_τ	C_0	C_{18}	$C_\tau = C_0(K_\tau/K_0)$	$\Delta = C_0 - C_\tau$	$S_f = C_{18} - C_\tau$	D*
chlorophyll-*c3*	3/Jul/2003	seawater	197	5	0.46	0.33	0.01	0.45	0.32	29.0
			198	5	0.46	0.32	0.01	0.45	0.31	31.2
	30/Jul/2003		606	25	1.41	1.41	0.06	1.35	1.35	0.00
			696	15	1.62	1.62	0.03	1.59	1.59	0.00
	4/Aug/2003		249	30	0.58	0.43	0.07	0.51	0.36	29.4
			163	15	0.38	0.38	0.03	0.35	0.35	0.00
	2/Oct/2003		1485	1080	1.52	1.40	1.11	0.41	0.29	29.0
			1410	1087	1.44	1.41	1.11	0.33	0.30	9.09
	6/Nov/2003		7734	7532	2.72	2.70	2.65	0.07	0.05	30.9
			7654	7543	2.73	2.72	2.69	0.04	0.03	25.3
peridinin	30/Jul/2003	seawater	43	0	2.29	1.72	0.00	2.29	1.72	24.3
			40	0	2.25	2.25	0.00	2.25	2.25	0.00
	4/Aug/2003		28	0	0.95	0.79	0.00	0.95	0.79	16.8
			56	0	1.20	0.90	0.00	1.20	0.90	25.0
	2/Oct/2003		562	453	2.16	2.15	1.74	0.42	0.41	2.39
			393	253	2.05	2.04	1.32	0.73	0.72	1.37
	6/Nov/2003		15	15	0.51	0.51	0.51	0.00	0.00	-
			16	16	0.51	0.50	0.51	0.00	-0.01	-

Table 4.4 (continued)

Pigments	Dates	Culture media	K_0	K_r	C_0	C_{18}	$C_r = C_0(K_r/K_0)$	$\Delta = C_0 - C_r$	$S_f = C_{18} - C_r$	D*
fucoxanthin	3/Jul/2003	seawater	405	5	4.34	4.34	0.06	4.33	4.33	0.41
			401	5	4.34	4.34	0.06	4.33	4.34	0.20
	30/Jul/2003		685	25	0.91	0.17	0.03	0.88	0.14	84.3
			775	15	0.91	0.91	0.02	0.89	0.89	0.00
	4/Aug/2003		745	0	6.35	5.25	0.00	6.35	5.25	17.3
			1251	185	6.53	5.74	0.97	5.56	4.34	14.3
	2/Oct/2003		2047	566	5.10	4.33	1.41	3.69	3.42	7.32
			1803	458	4.34	4.49	1.23	3.62	3.36	7.19
	6/Nov/2003		7749	7532	3.14	3.11	3.05	0.09	0.06	33.0
			7668	7543	3.12	3.10	3.07	0.05	0.03	39.3
	3/Jul/2003	chrysophytes	55097	39606	16.7	15.1	12.0	4.33	3.10	34.1
			55265	39774	16.9	15.1	12.2	4.33	2.94	38.0
	30/Jul/2003		1485	0	9.58	8.06	0.00	9.58	8.06	15.9
			1485	0	9.58	5.04	0.00	9.58	5.04	47.4
	4/Aug/2003		787	180	7.88	4.36	1.80	6.08	2.56	57.9
			952	180	9.05	4.33	1.71	7.34	3.02	58.9
	2/Oct/2003		1748	0	9.88	8.27	0.00	9.88	8.27	16.3
			1661	0	9.39	7.86	0.00	9.39	7.86	16.3
	6/Nov/2003		590	120	5.23	4.33	1.06	4.33	3.17	24.0
			575	110	5.12	4.33	0.98	4.33	3.24	21.7

Table 4.4 (continued)

Pigments	Dates	Culture media	K_0	K_r	C_0	C_{18}	$C_r = C_0(K_r/K_0)$	$\Delta = C_0 - C_r$	$S_f = C_{18} - C_r$	D*
19-hex-fucoxanthin	30/Jul/2003	seawater	606	25	0.66	0.66	0.03	0.63	0.63	0.00
			696	15	0.76	0.75	0.02	0.74	0.73	1.34
	4/Aug/2003		249	30	0.77	0.73	0.09	0.68	0.64	5.91
			163	15	0.01	0.01	0.00	0.01	0.01	0.00
	2/Oct/2003		1485	1080	0.80	0.73	0.58	0.22	0.15	32.1
			1410	1087	0.76	0.69	0.59	0.17	0.10	40.2
	6/Nov/2003		7734	7532	0.62	0.61	0.60	0.02	0.01	61.8
			7654	7543	0.61	0.61	0.60	0.01	0.01	0.00
alloxanthin	30/Jul/2003	seawater	331	155	0.37	0.33	0.17	0.20	0.16	20.3
			412	234	0.46	0.42	0.26	0.20	0.16	20.1
	4/Aug/2003		456	405	0.51	0.51	0.45	0.06	0.06	0.00
			510	203	0.57	0.44	0.23	0.34	0.21	37.9
	6/Nov/2003		175	45	0.32	0.30	0.08	0.24	0.22	8.41
			169	44	0.31	0.29	0.08	0.23	0.21	8.72

Table 4.4 (continued)

Pigments	Dates	Culture media	K_0	K_r	C_0	C_{18}	$C_r = C_0(K_r/K_0)$	$\Delta = C_0 - C_r$	$S_f = C_{18} - C_r$	D*
lutein	3/Jul/2003	seawater	730	134	0.84	0.71	0.15	0.69	0.56	19.0
			752	158	0.83	0.70	0.17	0.66	0.53	19.8
	30/Jul/2003		689	595	0.76	0.74	0.66	0.10	0.08	19.3
			734	689	0.81	0.80	0.76	0.05	0.04	20.1
	4/Aug/2003		925	123	1.02	0.84	0.14	0.88	0.70	20.4
			698	56	0.77	0.54	0.06	0.71	0.48	32.5
	2/Oct/2003		1486	1030	1.18	1.10	0.82	0.36	0.28	22.1
			1412	979	1.12	1.05	0.78	0.34	0.27	20.4
	6/Nov/2003		850	450	0.57	0.55	0.30	0.27	0.25	7.46
			830	430	0.56	0.53	0.29	0.27	0.24	11.1

Table 4.4 (continued)

Pigments	Dates	Culture media	K_0	K_r	C_0	C_{18}	$C_r = C_0(K_r/K_0)$	$\Delta = C_0 - C_r$	$S_r = C_{18} - C_r$	D*
chlorophyll-*a*	3/Jul/2003	seawater	1135	139	12.9	12.9	1.58	11.3	11.3	0.00
			1153	163	13.0	13.0	1.84	11.2	11.2	0.00
	30/Jul/2003		1748	775	5.05	4.40	2.24	2.81	2.26	19.6
			1961	938	4.37	4.00	2.14	2.33	1.86	20.2
	4/Aug/2003		2154	528	20.7	13.9	5.07	15.6	8.83	43.5
			2515	444	24.3	18.7	4.33	20.5	14.3	30.2
	2/Oct/2003		4095	2049	18.1	13.8	9.06	9.04	4.33	47.6
			3608	1690	17.2	13.1	8.06	9.14	5.04	44.3
	6/Nov/2003		8789	8042	13.9	13.7	12.7	1.18	0.98	16.9
			8683	8033	13.9	13.6	12.9	1.04	0.74	28.8
	3/Jul/2003	chrysophytes	55097	39606	57.1	47.8	41.1	16.1	6.75	57.9
			55265	39774	57.1	47.9	41.1	16.0	6.81	57.5
	30/Jul/2003		1485	0	27.9	23.8	0.00	27.9	23.8	14.5
			1485	0	27.9	12.3	0.00	27.9	12.3	55.9
	4/Aug/2003		787	180	23.3	14.3	5.33	18.0	9.37	47.9
			952	180	25.5	14.2	4.33	20.7	9.38	54.6
	2/Oct/2003		1748	0	30.1	25.0	0.00	30.1	25.0	16.9
			1661	0	28.6	23.8	0.00	28.6	23.8	16.8
	6/Nov/2003		590	120	20.1	19.0	4.09	16.0	14.3	6.87
			575	110	20.1	19.0	3.85	16.3	15.2	6.77

Table 4.5 A summary of mean degradation efficiencies of different phytoplankton pigments after ingestion by *P. avirostris* and *Paracalanus* spp.

P. avirostris

Pigments	Pigment degradation (%)	
	mean	range
chlorophyll-*c3*	15.1	1.72-28.3
peridinin	14.0	0.87-24.3
fucoxanthin	19.8	0.00-45.7
19-hex-fucoxanthin	13.4	1.83-25.7
alloxanthin	12.1	3.28-31.7
lutein	9.78	0.00-30.5
chlorophyll-*a*	24.3	0.00-68.8

Paracalanus spp.

Pigments	Pigment degradation (%)	
	mean	range
chlorophyll-*c3*	18.4	0.00-30.9
peridinin	11.8	0.00-25.0
fucoxanthin	26.7	0.00-58.9
19-hex-fucoxanthin	17.7	0.00-61.8
alloxanthin	15.9	0.00-37.9
lutein	19.2	7.46-32.5
chlorophyll-*a*	29.4	0.00-57.9

4.5 Clearance rates of *P. avirostris* and *Paracalanus* spp. in feeding experiments

Clearance rates of *P. avirostris* and *Paracalanus* spp. were calculated as in Section 3.2.5 (Table 4.6). C_2* for pigment concentrations was not corrected with fecal pigments. Some of the replicates were discarded (~ 5%). There were mainly two cases for discarding. Firstly, no ingestions occurred in the discarded replicates. These were due to the negative selectivity of the zooplankton on phytoplankton. One of examples was the negative selection of both *P. avirostris* and *Paracalanus* spp. on dinoflagellates. Secondly, the final pigment concentration was higher than the initial concentration due to a higher excretion rate of pigment than the ingestion rate. These might also be due to the negative selection of zooplankton on phytoplankton. The mean clearance rates calculated ranged from 0.02 mL h^{-1} based on peridinin to 1.02 ml/h based on cell counting for *P. avirostris* and from 0.01 mL h^{-1} based on peridinin to 1.55 mL h^{-1} based on 19-hex-fucoxanthin for *Paracalanus* spp. Figure 4.36 presents all the mean clearances rates. Further discussion on the implication of the differences in clearance rates will be presented Section 5.

Table 4.6 Clearance rates of *P. avirostris* and *Paracalanus* spp. calculated using data of total phytoplankton cell concentration and different phytoplankton pigment marker concentrations from HPLC analysis in laboratory feeding experiments.

Dates	Culture media	zooplankton	Cell conc. (cells mL^{-1})	Chl-*a* conc. (ng mL^{-1})	Clearance rates ($mL\ h^{-1}$)						
					cell count	HPLC					
						chl-*a*	Chl-*c3*	peridinin	fucoxanthin	19-hex-fucoxanthin	alloxanthin	lutein
3/Jul/2003	seawater	*P. avirostris*	500	12.4	1.80	0.07						
			528	12.5	1.70	0.07						
	chrysophytes		49650	53.3	1.68	0.13			0.08			
			49699	53.4	0.98	0.13			0.09			
	seawater	*Paracalanus*	474	12.9	1.94		0.31		4.07			0.16
		spp.	474	13.0			0.34		4.06			0.16
	chrysophytes		46926	52.3	0.31	0.16			0.31			
			47096	52.4	0.30	0.16			0.3			
30/Jul/2003	seawater	*P. avirostris*	806	5.05	0.20	0.00	0.01		1.55	0.01	0.03	
			1209	4.01	0.20	0.11	0.05			0.12	0.08	0.06
	chrysophytes		743	13.9								
			743	14.0								
	seawater	*Paracalanus*	1196	4.77	0.75	0.11			3.07	4.34	0.11	0.02
		spp.	1387	4.23	0.68	0.10			3.65	3.55	0.08	0.01
	chrysophytes		742	14.0								
			742	14.0								

4/Aug/2003	seawater	*Paracalanus*	1156	17.1	1.30	0.37	0.28			1.96		1.66
		spp.	1194	21.7	1.61	0.27			1.77	2.21	0.24	0.33
	chrysophytes		411	18.7	1.37	0.43			1.37			
			463	19.3	1.54	0.54			1.54			
2/Oct/2003	seawater	*P. avirostris*	3060	16.1	1.09	0.17	0.12	0.01	1.68	0.02		0.21
			3432	14.2	1.08	0.18	0.11	0.02	1.69	0.03		0.24
	chrysophytes		1828	29.5	1.11	0.20			1.71			
			1750	27.8	1.12	0.19			1.71			
	seawater	*Paracalanus*	2955	15.9	1.04	0.25	0.08		0.05	0.08		0.07
		spp.	2529	15.1	1.03	0.25	0.02		0.05	0.09		0.06
	chrysophytes		874	15.1	1.06	0.17			0.16			
			831	14.3	1.07	0.17			0.16			
6/Nov/2003	seawater	*P. avirostris*	8278	13.1	0.11	0.02	0.01		0.01	0.02	0.12	0.14
			8285	13.1	0.11	0.02	0.01		0.02	0.02	0.13	0.12
	chrysophytes		298	9.99	1.49	0.01			0.04			
			286	9.74	1.56	0.01			0.02			
	seawater	*Paracalanus*	8410	13.3	0.08	0.01	0.01		0.01	0.02	0.06	0.03
		spp.	8354	13.4	0.07	0.02	0.003	0.02	0.01	0.06		0.05
	chrysophytes		295	10.1	1.47	0.05			0.20			
			281	9.83	1.53	0.05			0.18			

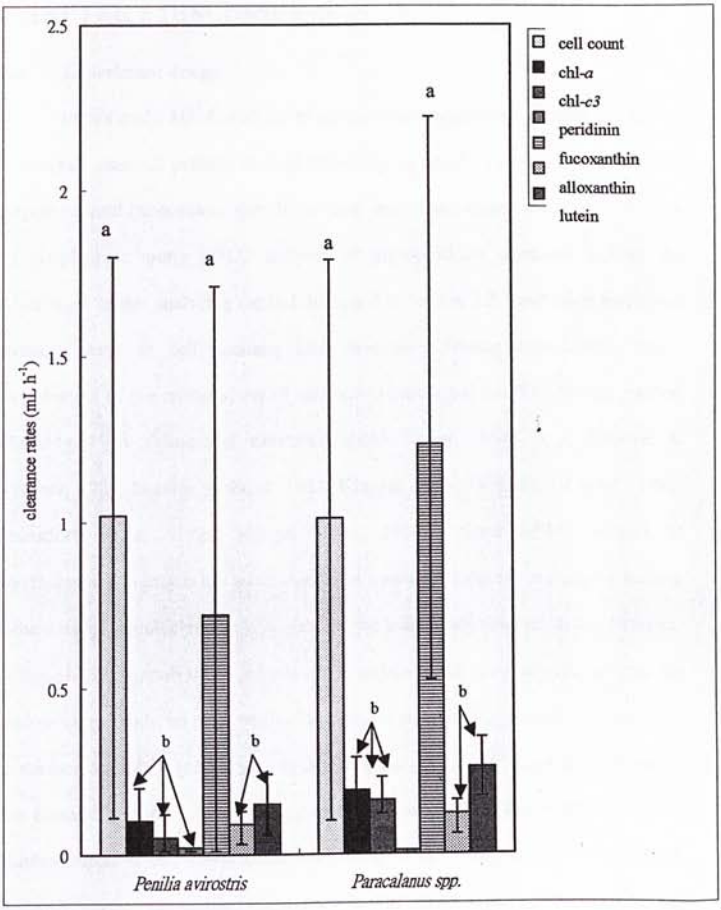


Figure 4.33 Clearance rates (mean ± range) of *P. avirostris* and *Paracalanus* spp. obtained from different pigment markers and cell counting method. (Data based on Table 4.8) (For each zooplankton, different letters represent significant difference at $P > 0.05$)

CHAPTER 5 DISCUSSIONS

5.1 Experiment design

In this study, HPLC analysis of phytoplankton pigments was used as a tool to investigate seasonal patterns in food selectivity by *Penilia avirostris, Pseudevadne tergestina, and Paracalanus* spp. In the past, many researchers have studied the diet of zooplankton using HPLC analysis of phytoplankton pigments due to the advantages of this analyzing method discussed in Section 2.2.4 and other traditional methods such as cell counting after laboratory feeding experiments, direct examination of gut contents, use of radioactive tracers and gut fluorescence method (Easterly, 1916; Schnack & Elbrächter, 1983; Turner, 1984a, b, c; Stoecker & Sanders, 1985; Huntley & Boyd, 1984; Kleppel, 1992, 1993; Poulet *et al.*, 1994; Jónasdóttir *et al.*, 1995; Kleppel *et la.*, 1996). Since HPLC analysis of phytoplankton pigments has advantages over traditional methods in studying feeding behaviour of zooplankton, it was used as the main study method in my research. Although HPLC analysis of phytoplankton pigments has some advantages over the traditional methods, its most serious limitation is pigment degradation which leads to underestimation of ingestion rates by zooplankton. In the early stage, researchers did not consider the implications of pigment degradation (Swadling & Marcus, 1994; Buffan-Dubau *et al.*, 1996; Quiblier-Llobéras *et al.*, 1996; Breton *et al.*, 1999). Recently, many researchers measured feeding rates with other methods and compared the results with those obtained by the HPLC method (e.g. Gasparini *et al.*, 2000).

Swadling & Marcus (1994), Buffan-Dubau *et al.*, (1996), Quiblier-Llobéras *et al.* (1996) and Breton *et al.* (1999) were among the first to use HPLC to measure the contents of phytoplankton pigments in zooplankton guts in order to estimate the feeding selectivity behavior of zooplankton. Their works were significant, but their

results were not corrected for pigment degradation. Other researchers used gut pigment analysis by HPLC together with other methods to study feeding of zooplankton in order to verify the results obtained by HPLC analysis of gut pigment. For example, Gasparini *et al.* (2000) used three complementary methods to evaluate *in situ* feeding activities of dominant copepod species of the Belgian coastal zone: gut pigment content analysis using HPLC, the ^{14}C tracer method and cell count experiments. Feeding selectivity of zooplankton was studied in the field and in laboratory feeding experiments. The results obtained by all three methods consistetly showed that prymnesiophyte *Phaeocystis globosa* is not an adequate food source for the spring copepod in the Belgian coastal zone. However, pigment degradations in zooplankton guts were not estimated. Studies on pigment degradation were done by many researchers (e.g. Lopez & Huntley, 1988; Penry & Frost, 1991; Head, 1992; Mayzaud & Razouls, 1992; Head & Harris, 1992, 1994; Suzanne *et al.*, 1998). However, most of the well studied pigments were chlorophylls. The degradation efficiencies for carotenoids obtained by different researchers were contradictory. Previous findings suggested that carotenoids might be more resistant to digestion than chlorophylls during passage through copepod guts (Kleppel *et al.*, 1988, 1991; Nelson 1989). However, later studies gave an opposite conclusion (Head & Harris, 1992, 1994). As a result, pigment degradation experiments were done in my research to further investigate the degradation of pigments such as peridinin & fucoxanthin and to verify the results obtained by HPLC analysis of phytoplankton pigments so that the amount of gut pigment in zooplankton collected in the field could be corrected.

In addition to measuring gut pigment contents, information on zooplankton feeding selectivity can also be obtained by measuring the change in phytoplankton pigment concentrations in water before and after zooplankton feeding. However, this

type of experiment is usually conducted in the laboratory and the behavior of zooplankton may be different as a result of handling. Nevertheless, using this method, the problem of pigment degradation can be solved. Examples of researchers using HPLC analysis of phytoplankton pigments in laboratory feeding experiments included Head & Harris (1994) and Meyer-Harms *et al.* (1999). This type of experiments was also carried out in my research for calculating the clearance rates for zooplankton on different phytoplankton.

5.2 Seasonal zooplankton feeding selectivity investigated by HPLC phytoplankton pigment analysis

5.2.1 Correlations between phytoplankton cell densities and pigment concentrations in water samples

The phytoplankton pigments chlorophyll-*c3*, peridinin, fucoxanthin, alloxanthin and lutein were used as signatures for prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green alga, respectively. In most cases, there were significant correlations between individual phytoplakton taxon and its signature pigment. However, it must be noted that significant correlation between dinoflagellate and its unambiguous pigment marker peridinin was found only at Station II. This was surprising because previous studies (cited in Chapter 2) have shown that peridinin was a marker for dinoflagellates such as *Prorocentrum* spp. and *Scrippsiella trochoidea* that are common in Tolo Harbour (Wright *et al.*, 1991; Jeffrey, 1997; Jeffrey & Vesk, 1997). The lack of significant correlation between dinoflagellate and peridinin at Station I could be due to the presence of taxa with low pigment level such as *Noctiluca scintillans*.

Poor correlations were found between chlorophyll-*a* and the densities of total phytoplankton, prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green algae. The reason for this might be the presence of phytoplankton, such as some dinoflagellates, which do not contain chlorophyll-*a*. The lack of correlation between chlorophyll-*a* and total phytoplankton showed that chlorophyll-*a* is not a good indicator of phytoplankton biomass if the phytoplankton is dominated by dinoflagellates.

5.2.2 Feeding selectivity of zooplankton on different phytoplankton groups

The guts of *Penilia avirostris* contained chlorophyll *c3*, peridinin,

fucoxanthin, alloxanthin and lutein, indicating that *P. avirostris* ate prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green algae. The guts of *Paracalanus* spp. contained chlorophyll *c3*, fucoxanthin, alloxanthin and lutein, suggesting that *Paracalanus* spp. was feeding on prymnesiophytes, diatoms, cryptomonads and green algae. However, if the amount of certain groups of phytoplankton eaten was small, the phytoplankton pigments might be totally digested and could not be detected. Therefore, the absence of a particular phytoplankton pigment marker does not mean that the corresponding phytoplankton group was not eaten.

Nevertheless, the results for *P. avirostris* showed that cryptomonads were selected positively at both stations and diatoms and green alga at one station. Therefore, my result supported previous findings that *P. avirostris* preferred diatoms (Gore, 1980; Lipej *et al.*, 1997; Paffenhöfer and Orcutt, 1986; Turner *et al.*, 1988; Kim *et al.*, 1989). *P. avirostris* ingest food particles ranging from >2.5 to 100 μm (Katechakis *et al.*, 2004). Ingestion of cryptomonads and green algae by *P. avirostris* has not been reported in the literature. Nevertheless, these groups might be eaten due to their abundance and small size (~5μm). Several reasons could explain the negative selection for dinoflagellates and prymnesiophytes by *P. avirostris*. First, many of the dinoflagellates (e.g. *Ceratium* spp.) in Tolo Harbour were probably too large (~100-180μm) to be ingested by *P. avirostris*. Second, some dinoflagellates such as *Prorocentrum lima* and *Gymnodinium* spp. may be toxic or unpalatable to zooplankton (Yamaji, 1984; Okaichi *et al.*, 1989; Yasuwo *et al.*, 1990; Hallegraeff, 1992). Third, dinoflagellates were rarely eaten by *P. avirostris* because they were not abundant in Tolo Harbour except in spring. Finally, dinoflagellates were mobile and have the ability to avoid capture by filter feeders such as *P. avirostris* (Katechakis *et al.*, 2004). However, the dinoflagellate *Prorocentrum* was frequently detected in guts of *P. avirostris* when it was the dominant dinoflagellate in the

phytoplankton. The small size of *Prorocentrum* spp. (~23μm) was suitable for *P. avirostris* (Gore, 1980; Paffenhöfer & Orcutt, 1986; Turner *et al.*, 1988; Kim *et al.*, 1989a; Lipej *et al.*, 1997). In addition, some dinoflagellates have a high nutritional value (Hitchcock, 1982; Weisse & Scheffel-Möser, 1990; Stoecker & Capuzzo, 1990). Therefore, peridinin was detected in guts of *P. avirostris* in February at both sampling stations and in March & June at Station II when *Prorocentrum* spp. was abundant.

Paracalanus spp. at both stations selected diatoms but appeared to reject prymnesiophytes, dinoflagellates and green alga. Reasons for selectivity of *Paracalanus* spp. on diatoms and dinoflagellates might be similar to *P. avirostris*. However, *Paracalanus* spp. avoided dinoflagellates even when *Prorocentrum* spp. was dominant. Cryptomonads were eaten by *Paracalanus* spp. according to their proportion in the phytoplankton. Very little information on the feeding selectivity of *Paracalanus* spp. is available in the literature.

Diatoms were eaten by *P. avirostris* throughout the year regardless of their abundance in the phytoplankton. This showed that *P. avirostris* had a strong preference on diatoms. However, selectivity of *P. avirostris* on cryptomonads and green alga changed with their proportion in the phytoplankton. In March and August at Station I, the absence of alloxanthin in the guts of *P. avirostris* indicated that cryptomonads were not eaten even though they were found in the sea. Dinoflagellate density of 203 cells mL^{-1} (Figure 4.6) could have an effect on the feeding selectivity of *P. avirostris* at Station I in March. In August, diatom density was higher than 8,000 cells mL^{-1}. *P. avirostris* might have enough preferred food so they ignored the less preferred cryptomonads.

Feeding selectivity of *Paracalanus* spp. on diatoms and dinoflagellates did not change so much temporally with the changes in abundance and dominance of

these two phytoplankton groups. On the other hand, prymnesiophytes and green alga were detected in guts of *Paracalanus spp.* only in some months. Prymnesiophytes were detected in guts of *Paracalanus spp.* in May at Station I. Proportions of prymnesiophytes in guts of *Paracalanus spp.* were higher than those in seawater. Low concentrations of other phytoplankton (Figure 4.5) might force *Paracalanus* spp. to feed on the less preferred food. Green algae were detected in guts of *Paracalanus spp.* in June at both stations and in January at Station II, but the proportions of green alga in guts of *Paracalanus spp.* from these three samples were comparable to or slight less than those in seawater.

5.2.3 Feeding selectivity of *Pseudevadne tergestina*

Feeding selectivity of *P. tergestina* in Tolo Harbour was investigated when the density of *P. tergestina* was high enough for easy sampling in October 2003. Diatoms were detected in guts of *P. tergestina*. This agreed with previous finding by Kim *et al.* (1989a) that *P. tergestina* fed on diatoms, particularly centric diatoms such as *Chaetoceros* spp. While it could not be known from HPLC analysis what types of diatoms were eaten, diatoms in Tolo Harbour was dominated by centric diatoms on the sampling day. Although my result indicated ingestion of diatoms by *P. tergestina*, it was difficult to tell whether *P. tergestina* had also eaten other phytoplankton as the undetected pigments specific to phytoplankton taxa other than diatoms might be completely degraded in guts of *P. tergestina*.

5.3 Feeding experiments for investigating pigment degradation in guts of zooplankton

5.3.1 Principle

The principle of the pigment degradation experiments has been outlined in various studies (e.g. Lopez & Huntley, 1988; Penry & Frost, 1991; Head, 1992; Mayzaud & Razouls, 1992; Head & Harris, 1992, 1994; Suzanne *et al.*, 1998). It assumed that the amount of pigments in water before incubation was similar to the amount of pigment in water after incubation together with the amount of pigment in fecal materials if there was no pigment degradation. Therefore, decrease in total pigment after incubation was due to pigment degradation. The assumption was that the amount of pigment in zooplankton gut did not change after feeding. The assumption was supported in my experiment when fluorometric measurement showed no change in chlorophyll-*a* concentrations *in* zooplankton guts before and after feeding. Pigment degradation efficiencies were used for correcting the pigment amounts in *in situ* zooplankton samples.

5.3.2 Degradation for different pigments in guts of *P. avirostris* and *Paracalanus* spp.

The mean pigment degradation efficiencies of *P. avirostris* ranged from 9.78% for lutein to 24.3% for chlorophyll-*a*. The mean pigment degradation efficiencies of *Paracalanus* spp. ranged from 11.75% for peridinin to 29.37% for chlorophyll-*a*. Degradation of chlorophyll-*a* in copepods varied from 10 to 90% with food concentrations and previous feeding history (Conover et al., 1986; Head, 1988; Lopez et al., 1988; Lopez & Huntley, 1988; Penry & Frost, 1991; Head, 1992; Mayzaud & Razouls, 1992; Head & Harris, 1992, 1994; Suzanne *et al.*, 1998). Degradation rates recorded in my study were within this range. Pigment losses in

guts of zooplankton were corrected using results from pigment degradation experiments. Of course, there was the assumption that pigment degradation rates obtained in the laboratory could be applied to natural conditions.

My findings are different from those of Head and Harris (1994) who found that carotenoids were completely destroyed in zooplankton guts. However, there were findings that carotenoids might be more resistant to digestion than chlorophylls during passage through copepod guts (Kleppel et al., 1988, 1991; Nelson, 1989).

5.4 Clearance rates of *P. avirostris* and *Paracalanus* spp.

5.4.1 *P. avirostris*

The mean clearance rate for *P. avirostris* on chlorophyll-*a* based on HPLC measurement was 0.1 mL h^{-1}. Clearance rate for *P. avirostris* was previously investigated in Tolo Harbour by Wong *et al.* (1992). Mean clearance rates in June and November were 0.09 and 0.03 mL /h^{-1} respectively. My results were comparable to those of Wong *et al.* (1992). However, clearance rates obtained in this study were ~10-20 times lower than those reported by Paffenhöfer & Orcutt (1986) and Turner *et al.* (1988), but animals used in those previous studies were collected in oceanic waters where food concentrations were considerably lower (≤0.01 mm^3 L^{-1}). In addition, animals feeding on pure algal diets in the laboratory have been kown to exhibit feeding rates higher than those observed under natural conditions (Dagg & Grill, 1980; Nicolajsen *et al.*, 1983).

My study of *P. avirostris* feeding on different phytoplankton revealed that mean clearance rates were highest on diatoms (Figure 4.53). The mean clearance rate on fucoxanthin was 0.72 mL h^{-1}. Athough fucoxanthin was contained in diatoms as well as prymnesiophytes, mean clearance rates on chlorophyll *c3* (0.05 mL h^{-1}) and 19-hexa-fucoxanthin (0.04 mL h^{-1}) that were specific for prymnesiophytes were low. Therefore, diatoms were responsible for the high mean clearance rate of fucoxanthin. Mean clearance rates on lutein (0.15 mL h^{-1}) and alloxanthin (0.09 mL h^{-1}) were the second and third highest. Mean clearance rates on peridinin (0.02 mL h^{-1}) were the lowest. The clearance rates on different phytoplankton indicated the selectivity of *P. avirostris* on them. Therefore, *P. avirostris* preferred diatoms, green alga, cryptomonads, prymnesiophytes and dinoflagellates in decreasing order. This result matched the result obtained *in situ* by HPLC gut pigment analysis in Section 4.2.4.

5.4.2 *Paracalanus* spp.

Mean clearance rate for *Paracalanus* spp. on diatoms based on HPLC analysis was high (1.23 mL h^{-1}) relative to other phytoplankton. This agreed with the result of strong selection on diatoms by *Paracalanus* spp. obtained by gut pigment analysis using HPLC. The low mean clearance rates on dinoflagellates (0.01 mL h^{-1}) and green alga (0.26 mL h^{-1}) also agreed with the results from gut pigment analysis by HPLC carried out in Section 4.2.4. From the result of seasonal feeding selectivity by HPLC analysis of phytoplankton pigment, *Paracalanus* spp. neither selected for or against cryptomonads. In the feeding experiments, mean clearance rate for *Paracalanus* spp. on cryptomonads was lower than that on green algae. This might suggest that *Paracalanus* spp. also did not prefer cryptomonads.

Ingestion rates obtained in my study were higher than those in the literature. Wang et al. (1998) reported that the ingestion rate for copepods with sizes of 200-500 mμm including *Paracalanus parvus* was 4.00-12.65 ng chl-*a* ind^{-1} day^{-1}. However, the average ingestion rate for *Paracalanus* spp. on phytoplankton in natural seawater in my feeding experiment was 43.93 ng chl-*a* ind^{-1} day^{-1}.

5.5 Limitations of HPLC analysis of phytoplankton pigments

Although HPLC analysis of phytoplankton pigments have a lot of advantages in the study of feeding selectivity of zooplankton as discussed in Section 2.2.4, there were some limitations. In my study, phytoplankton compositions were determined by microscopic examination. Only this method could truly identify the phytoplankton species in the sea. HPLC analysis of phytoplankton pigments could indicate the abundance of phytoplankton at approximately the class level. It could not identify the phytoplankton to the species level. If the feeding selectivity of zooplankton on a specific phytoplankton species is to be investigated, plankton identification and enumeration using microscope must be done. In addition, feeding selectivity of zooplankton determined by HPLC pigment analysis will not be reliable enough without supports by other experiements due to the problem of pigment degradation. As a result, pigment degradation experiments were carried out in my study. Corrections of pigment degradations in zooplankton guts after HPLC gut pigment analysis and further data on the clearance rates for zooplankton on different phytoplankton groups could make the results of *in situ* zooplankton feeding selectivity by HPLC analysis of phytoplankton pigments becoming more reliable. From my results, pigment degradation did not have a great impact on the results of feeding selectivity of zooplankton obtained by HPLC analysis of gut pigments as the conclusions drawn from results of feeding selectivity of zooplankton obtained by HPLC analysis with or without pigment degradation correction were the same. However, the application of pigment degradation results in correcting the pigment degradations in *in situ* zooplankton samples might not be appropriate. Pigment degradation in zooplankton guts can be affected by food composition and feeding history of zooplankton (Penry & Frost, 1991; Head, 1992; Head & Harris, 1992). The extent of pigment degradation *in situ* might be totally different from that in the

laboratory. Therefore, other experiments should be carried out in addition to HPLC analysis of phytoplankton pigments if HPLC analysis of phytoplankton pigments is used to study feeding selectivity of zooplankton.

5.6 Environmental events related to feeding selectivity of zooplankton in Tolo Harbour

5.6.1 Energy transfer in trophic level

Both *P. avirostris* and *Paracalanus* exhibited selective feeding, preferring to eat diatoms. This implied that the energy going to the higher trophic level was not limited by the selective feeding of zooplankton. Both zooplankton species selected against dinoflagellates. Red tides due to dinoflagellate blooms were common in Tolo Harbour (Hodgkiss & Ho, 1997). My results suggest that the growth of dinoflagellates in Tolo Harbour would not be controlled by zooplankton grazing.

5.6.2 Abilities of *P. avirostris* and *Paracalanus* spp. to control red tides in Tolo Harbour

Most of the red tides in Tolo Harbour were caused by dinoflagellates (Wu & Wong, 1987; Lam & Ho, 1989; Hodgkiss & Ho, 1997; HKEPD, 2001). Red tides can be toxic or non-toxic depending on the red tide caustive species. However, non-toxic red tides can also be harmful. For example, red tide blooms of *Noctiluca scintillans*, *Scrippsiella trochoidea* and *Trichodesmium ervthraeum* leading to anoxia may kill fish and marine invertebrates (Holmes & Lam, 1985; Hallegraeff, 1992; Hallegraeff, 1993; Matthews & Pitcher, 1996). In addition, red tides may affect local mariculture, fishing industry, recreational interest and human health. As a result, finding methods to control red tides is important. Biological control such as depending on grazing by zooplankton on red tide species is one of the possible methods. In Tolo Harbour, the abundant zooplankton - *P. avirostris* and *Paracalanus* spp. selected for diatoms from my results. They might be able to control algal blooms of diatoms. However, for red tides due to dinoflagellate blooms, both of the species might not be able to control them. *P. avirostris* grazed on the dinoflagellate,

Protocentrum spp., suggesting that *P. avirostris* might be able to control this kind of red tides.

CHAPTER 6 CONCLUSIONS

The major conclusions are:

1. HPLC analysis of phytoplankton pigments could be used for in situ investigation of feeding selectivity of zooplankton if enough supplementary experiments such as pigment degradation were done to verify pigment concentrations obtained by HPLC analysis of phytoplankton pigments.

2. Chlorophyll *c3*, peridinin, fucoxanthin, alloxanthin and lutein could be used as a marker for prymnesiophytes, dinoflagellates, diatoms, cryptomonads and green alga respectively. Correlation between these phytoplankton pigments and the densities of the corresponding phytoplankton groups showed that these pigments were indicators of the corresponding phytoplankton groups.

3. *P. avirostris* showed strong selection for diatoms, cryptomonads and green alga, but selected against dinoflagellates and prymnesiophytes.

4. *P. avirostris* exhibited strong selection for dinoflagellates only when the dinoflagellate community was dominated by *Protocentrum* spp. Selectivity by *P. avirostris* on dinoflagellates was weak when the dinoflagellate community was dominated by *Scrippsiella* spp. and *Noctiluca scintillans*.

5. *Paracalanus* spp. showed strong selection for diatoms, but selected against prymnesiophytes, dinoflagellates and green alga.

6. *P. tergestina* showed strong selection for diatoms, but selected against prymnesiophytes, dinoflagellates, cryptomonads and green alga.

7. Pigment degradation occurred in zooplankton guts. However, the extents of degradations varied among different pigments, ranging from 9.8% for lutein to 24.3% for chlorophyll-*a* in *P. avirostirs* and 11.8% for peridinin to 29.4% for chlorophyll-*a* in *Paracalanus* spp.

8. Feeding selectivities of *P. avirostris* and *Paracalanus* spp. in laboratory

feeding experiments were similar to that in the field.

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ACADEMIC ACHIEVEMENT

Manuscript in preparation

Study of selective feeding by the marine cladoceran *Penilia avirostris* using HPLC analysis of phytoplankton marker pigments

(Journal of Plankton Research)

Paper presented

Study of selective feeding by the marine cladoceran *Paracalanus* spp. using HPLC analysis of phytoplankton marker pigments

(Symposium on Marine Biology and Biotechnology, 20-24 April, 2004)

Poster to be presented

Study of selective feeding by the marine cladoceran *Penilia avirostris* using HPLC analysis of phytoplankton marker pigments

(The XIXth International Congress of Zoology, 23-27 August, 2004)